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(54) Title: BUFFER/ADDITIVES ELECTROLYTE COMBINATIONS FOR ELECTROKINETIC CHROMATOGRAPHY

(57) Abstract

The invention is an electrolyte comprising a buffer/additive combination for use in electrokinetic chromatography ("EKC"). The EKC buffer/additive combinations of this invention make practical the use of larger diameter capillaries, therefore allowing higher resolution, faster analysis time, and improved detectability compared to buffer/additive combinations currently used in EKC. The invention is a capillary electrophoresis electrolyte for separating analyte components of interest contained in a substantially aqueous sample, comprising a substantially aqueous phase, a protonated additive dispersed in the substantially aqueous phase, and a free polyamine dispersed in the substantially aqueous phase. The substantially aqueous phase may also include an organic modifier such as methanol or acetonitrile. The protonated additive may be a surfactant, and for chiral separations, a chiral surfactant. The invention may also be used with an achiral surfactant. A preferred embodiment is the chiral surfactant (S)-N-dodecoxycarbonylvaline. The free polyamine refers to an amine molecule having two or more amine moieties, such as a diamine. A preferred embodiment of a diamine is bis-tris propane.

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Buffer/Additives Electrolyte Combinations for Electrokinetic Chromatography

This application is a continuation in part of application Ser. No. 08/503,462, filed July 18, 1995.

1. Field of the Invention

The invention is in the field of electrokinetic chromatography. In particular, the invention relates to improved buffer/additive combinations for electrokinetic chromatography which afford fast, high resolution separations with increased detectability.

2. Description of the Prior Art

Although a relatively new addition to the field of separations techniques, capillary electrophoresis ("CE") is already being used to separate a wide variety of solutes. Separations are performed in thin glass capillaries, as opposed to the 2-dimensional surfaces (such as gels or paper) which have traditionally been used in electrophoresis. CE has the ability to perform fast, high resolution separations in a simple experimental apparatus. Fast, high resolution separations result from the high efficiencies afforded by this technique.

High efficiency is obtained in CE because under ideal conditions, the sole source of band broadening is longitudinal diffusion (*Jorgenson, J.W. and Lukas, K.D.* Analytical Chemistry, 1981, **(53)** 1298-1302). Efficiency in separations systems is commonly expressed as theoretical plates (N). The larger the value of N, the higher the efficiency of the system. In capillary electrophoresis, under conditions where diffusion is the sole source of band broadening, the expression for N was derived by Jorgenson (*Jorgenson, J.W. and Lukas, K.D., supra*):

$$N = \frac{\mu V l}{2DL} \qquad \text{equation 1}$$

where μ is the solute's apparent mobility, V is the applied voltage, l is the length of capillary from injection to detection, D is the solute's diffusion coefficient, and L is the total capillary length.

Equation 1 predicts that by increasing the applied voltage, higher efficiency (and therefore higher resolution) will be obtained. Furthermore, higher voltages cause the solute to move through the capillary at greater velocity, resulting in faster

separation. Therefore, in theory, increasing the voltage will lead to faster analysis time and higher resolution. However, there is a limit to how high the voltage can be increased before resolution begins to degrade (*Knox, J.H.*, Chromatographia, **26:** 329-333 (1988); *Grushka, E., McCormick, R.M. and Kirkland, J.J.*, Analytical Chemistry **61:** 241-245 (1989) . This limit occurs when the heat which is generated during application of the separation voltage causes thermal gradients in the capillary. These thermal gradients lead to band broadening and loss of resolution. To understand both how thermal gradients are generated and how they generate a mechanism for band broadening and subsequent loss of resolution, we will review the basic theory.

As the voltage increases, the current also increases, as defined by Ohm's Law:

$$V = IR \qquad \text{equation 2}$$

where V is the voltage, I is the current, and R is the electrical resistance of the capillary. Temperature gradients in the capillary are related to the heat generation rate or power density Q (*Nelson, R.J., Paulus, A., Cohen, A.S., Guttman, A. and Karger, B.L.*, Journal of Chromatography, **480**: 111-127 (1989):

$$Q = \frac{VI}{\pi r^2 L} \qquad \text{equation 3}$$

where r is the capillary internal bore radius and L is the total capillary length. Thermal gradients exist when there is a difference in temperature between the buffer in the center of the capillary and the buffer near the wall. Since heat flows from warmer to cooler bodies, the temperature at the capillary wall will be lower because it is in contact with the cooler surrounding environment. Such temperature gradients lead to viscosity gradients within the capillary, with the viscosity being lowest in the center of the tube (where the buffer is warmest). Since the electrophoretic mobility of a solute is inversely proportional to the viscosity of the electrolyte, a solute molecule in the center of the tube will have a higher velocity than a solute molecule near the wall. This non-uniform velocity profile causes band broadening, which is deterimental to overall performance.

For a given electrolyte and capillary length, thermal gradients are proportional to the square of the capillary radius and the square of the applied voltage. To maximize V (and therefore analysis speed and resolution) capillaries with very small internal diameters must be employed (i.e., 1 μm) to increase heat dissipation so that thermal

gradients are not a substantial factor. However, small diameter capillaries lead to poor detectability when using on-column UV detection. The present state of the art in CE is to use capillaries with internal diameters of 50 or 75 μm to improve detection while keeping the band-broadening effect of the thermal gradients at an acceptable level.

Another way to reduce the deleterious effects of temperature gradients is to use electrolytes with low electrical conductivity (k). The most common electrolytes used in CE are prepared with inorganic salts as the buffering agents, such as sodium phosphate, sodium borate, etc. Because these salts dissociate completely in aqueous solution into highly mobile inorganic ions, the resulting electrolytes have high electrical conductivities. Lower concentrations of the salt can be used, but buffering capacity is reduced. Recently, the use of electrolytes prepared with zwitterionic buffers has gained acceptance in CE (*Weinberger, R.*, "Practical Capillary Electrophoresis", Academic Press, San Diego, CA, Chapter 2, pp. 37-39 (1993)). Such buffers have excellent capacities but low conductivities when used at their pI (where their net charge is zero). Using these buffers, large voltages can be applied for fast separations without the corresponding temperature gradients, band broadening, and subsequent resolution losses afforded by equivalent concentrations of non-zwitterionic buffers.

Electrokinetic chromatography (EKC), believed to be first reported by Terabe, is a subset of capillary *electrophoresis (Terabe, S., Otsuka, K., Ichikawa, K., Tsuchiya, A. and Ando, T.*, Analytical Chemistry, **56**: 111-113 (1984). In EKC, analytes partition between the bulk aqeous buffer and an additive. EKC electrolytes consist of a buffering agent and an additive which can interact with the solutes. Additives which have been used in EKC include micelles (MEKC or MECC; *Terabe, S. et al., supra*), cyclodextrins (*Terabe, S., Ozaki, H., Otsuka, K. and Ando, T.*, Journal of Chromatography, **332**:211-217 (1985)), polymer ions (*Terabe, S., Isemura, T.*, Analytical Chemistry, **62**: 652-656 (1990)), and proteins (*Yang, J. and Hage, D.S.*, Analytical Chemistry, **66**: 2719-2725 (1994). Resolution of two analytes is achieved in EKC by one or both of the following mechanisms:

1) differences in their mobilities in the bulk aqeous phase (capillary zone electrophoresis), and/or

2) differences in their partitioning between the bulk aqeous phase and the additive, with the further requirement that the mobility of the analyte-additive complex is different from the mobility of the analyte in the bulk aqeous phase.

The second mechanism requires the existence of a "migration window" in EKC. The migration window is the length of time between the point in time at which a neutral species elutes with no partitioning (t_0), and the elution time of an analyte compound which completely partitions into the additive. For instance, MEKC is usually performed with sodium dodecyl sulfate (SDS) micelles. SDS micelles are anionic and have an electrophoretic mobility towards the anode. Uncoated fused silica capillaries are typically used in MEKC, and a bulk electroosmotic flow toward the cathode is produced at pHs > 2.0. Above pH 6.0, the electroosmotic velocity is usually faster than the electrophoretic velocity of the SDS micelles, causing the micelles to have a net movement toward the cathode. This situation leads to a migration window, which for neutral analytes, is defined by the electroosmotic flow marker (no partitioning) and the micelle marker (complete partitioning). All neutral analytes must migrate between these two boundaries.

The existence of a migration window leads to an additional term in the resolution equation for MEKC compared to the standard resolution equation for chromatography. As developed by Terabe (*Terabe, S., Otsuka, K. and Ando, T.* Analytical Chemistry, 1985, (57) 834-841), the resolution equation for neutral analytes in MEKC is:

$$Rs = \left(\frac{N^{\frac{1}{2}}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{k_2 + 1}\right)\left(\frac{1 - \frac{t_0}{t_{mc}}}{1 + \frac{t_0}{t_{mc}} k_1}\right) \qquad \text{equation 4}$$

where N is the theoretical plate count, α is the selectivity term, k_1 and k_2 are the capacity factors for the two analytes, t_0 is the electroosmotic flow time, and t_{mc} is the micelle marker time.

The last term in equation 4 is known as the migration window term. Under conditions where the micelle and electroosmotic mobilities are directed toward the same electrode, reduction of the electroosmotic flow will lead to an increase in resolution. Therefore, MEKC electrolytes which lead to low electroosmotic flow are advantageous from a resolution standpoint.

The majority of MEKC separations have employed sodium dodecyl sulfate as the micelle forming agent. Like other forms of CE, the most common buffering agents are inorganic salts like sodium phosphate and sodium borate. Electrolytes prepared with these buffering agents are desirable from a resolution standpoint, since their high ionic strength reduces electroosmotic flow (Weinberger, R. *supra*). However, the electrolytes have high conductivity due to the high concentrations of mobile inorganic ions. To perform fast, high resolution separations with such electrolytes, capillary internal diameters of 50 and 75 μm are employed. The tradeoff is lower detectability when using on-column UV detection. The use of 100 μm capillaries in MEKC has been shown to lead to detection limits and limits of quantitation ten times lower than with 75 μm capillaries when all other variables (i.e., injection time, voltage, etc.) are kept constant (*Thomas, B.R., Fang, X.G., Chen, X., Tyrrell, R.J. and Ghodbane, S.* Journal of Chromatography, 1994, (657) 383-394). The improvement was attributed to higher injected amount and increased path length with on-column UV detection. However, the improvement in detectability came at the price of some sacrifice in resolution.

MEKC electrolytes using SDS as the surfactant have been prepared with zwitterionic buffering agents such as 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) (*Weinberger, R., Sapp, E. and Moring, S.*, Journal of Chromatography, 1990, (516) 271-279). However, there is still a large concentration of sodium present from the SDS. Furthermore, electrolytes prepared with a zwitterionic buffer at a given concentration in MEKC have lower resolution than those prepared with the same concentration of an inorganic salt. The electroosmotic flow is much faster with low ionic strength electrolytes, so the migration window term in the resolution equation is decreased.

The present state of the art of MEKC electrolytes is limited by the tradeoff which exists between good detectability and good separation performance. There is a need for an MEKC electrolyte that would have low conductivity so that large capillary i.d.s could be employed (i.e., 100 μm) for improved detectability and also allow the application of high voltages for fast, high resolution separations. Furthermore, it should give EOF rates similar to those now obtained with high conductivity electrolytes.

Summary of the Invention

The invention is directed to an electrolyte comprising a buffer/additive combination for use in electrokinetic chromatography ("EKC"). The EKC buffer/additive combinations of this invention make practical the use of larger diameter capillaries, therefor allowing higher resolution, faster analysis time, and improved detectability compared to buffer/additive combinations currently used in EKC.

The invention is also directed to a capillary electrophoresis electrolyte for separating analyte components of interest contained in a substantially aqueous sample, comprising a substantially aqueous phase, a protonated additive dispersed in the substantially aqueous phase, and a free polyamine dispersed in the substantially aqueous phase. The substantially aqueous phase may also include an organic modifier such as methanol or acetonitrile. The protonated additive may be a surfactant, and for chiral separations, a chiral surfactant. The invention may also be used with an achiral surfactant. A preferred embodiment is the chiral surfactant (S)-N-dodecoxycarbonylvaline. The free polyamine refers to an amine molecule having two or more amine moieties, such as a diamine. A preferred embodiment of a diamine is bis-tris propane.

The invention is also directed to a method for separating analyte components of interest contained in a substantially aqueous sample comprising the step of contacting the sample with an effective amount of a combination of a protonated additive and a free amine, under electrokinetic chromatographic conditions, the combination comprising a protonated additive dispersed in the substantially aqueous phase, and a free polyamine dispersed in the substantially aqueous phase.

The invention is also directed to a capillary electrophoresis electrolyte for separating analyte components of interest contained in a substantially aqueous sample, comprising a substantially aqueous phase, a protonated additive dispersed in the substantially aqueous phase, and a free polyamine dispersed in the substantially aqueous phase, the free polyamine having the following structural formula:

$$R' - NH - \left[CH_2 \right]_a - NH - R$$

wherein

a is from about 1 to 8 methylenes; and

R and R' are the same or different and may be alkyl, alkenyly or alkynyl substituents, branched or straight chain, substituted or unsubstituted, having from about 1 to 8 carbons, and may have one or more hydrophilic moities such as hydroxy, sulfhydryl, or amine.

Therefore, it is an object of the invention to provide a low-conductivity buffer useful in electrokinetic chromatography.

It is another object of the invention to enhance detectability by making practical the use of larger diameter capillary columns for electrokinetic chromatography.

These and other objects and advantages of the invention will become apparent in the following detailed description of the preferred embodiments, in conjunction with the drawings.

Brief Description of the Drawings

FIG. 1 is the chemical structure of (S)-N-dodecoxycarbonylvaline, the surfactant used for the MEKC separations.

FIG. 2 are chromatograms showing the separation of 1% (-)-ephedrine in the presence of (+)-ephedrine using 25 mM (S)-N-dodecoxycarbonylvaline and 25 mM Na_2HPO_4/25 mM $Na_2B_4O_7$, pH 8.5, in a 100 μm i.d. capillary, with hydrostatic injection times of 2 and 5 seconds.

FIG. 3 are chromatograms showing the separation of 1% (-)-ephedrine in the presence of (+)-ephedrine using 25 mM (S)-N-dodecoxycarbonylvaline and 50 mM bis-tris propane, pH 8.5, in a 100 μm i.d. capillary, with hydrostatic injection times of 2 and 5 seconds.

FIG. 4 is a chromatogram showing the separation of 0.1% (-)-ephedrine in the presence of (+)-ephedrine using 25 mM (S)-N-dodecoxycarbonylvaline and 50 mM bis-tris propane, pH 8.5, in a 100 μm i.d. capillary.

FIG. 5 is the chemical structure of bis-tris propane.

FIG. 6A is a chromatogram showing the separation of norephedrine enantiomers using 25 mM (S)-N-dodecoxycarbonylvaline and 25 mM Na_2HPO_4/25 mM $Na_2B_4O_7$, pH 8.5, in a 50 μm i.d. capillary with 15 kV applied voltage.

FIG. 6B is a chromatogram showing the separation of norephedrine enantiomers using 25 mM (S)-N-dodecoxycarbonylvaline and 50 mM bis-tris propane, pH 8.5, in a 50 μm i.d. capillary with 15 kV applied voltage.

FIG. 7 is a chromatogram showing the separation of norephedrine enantiomers using 25 mM (S)-N-dodecoxycarbonylvaline and 50 mM bis-tris propane, pH 8.5, in a 50 μm i.d. capillary with 30 kV applied voltage.

FIG. 8 are the chemical structures of polyamines other than bis-tris propane demonstrated to be useful in this invention.

FIG. 9 is a chromatogram showing the baseline separation of metoprolol enantiomers and was obtaiend using an electrolyte containing 25 mM (S)-N-dodecoxycarbonylvaline and 25 mM N,N'-bis(2-aminoethyl)-1,3-propanediamine.

FIG. 10 is a chromatogram showing the baseline separation of benzoin enantiomers and was obtained using an electrolyte containing 25 mM (S)-N-dodecoxycarbonylvaline and 50 mM pentrol.

FIG. 11 is a chromatogram showing the baseline separation of norphenylephrine enantiomers and was obtained using an electrolyte containing 25 mM (S)-N-dodecoxycarbonylvaline and 15 mM pentaethylenehexamine.

FIG 12 is a chromatogram of the baseline separation of metoprolol enantiomers and was obtained using an electrolyte containing 25 mM (S)-N-dodecoxycarbonylvaline and 20 mM 4,7,10-trioxa-1,13-tridecanediamine.

FIGS. 13A-E are the chemical structures of the additives, which include two anionic cyclodextrin derivatives (carboxymethylated-β-cyclodextrin and succinylated-β-cyclodextrin), a chiral crown ether (18-crown-6-tetracarboxylic acid), and two achiral surfactants (lauric acid and dodecyl sulfate).

FIG. 14A is a chromatogram of the separation of the enantiomers of verapamil and was obtained using an electrolyte containing 0.1% (w/v) carboxymethyl-β-cyclodextrin and 25 mM bis-tris propane.

FIG. 14B is a chromatogram of the separation of the enantiomers of verapamil and was obtained using an electrolyte containing 0.1% (w/v) carboxymethyl-β-cyclodextrin and 25 mM sodium tetraborate.

FIG. 15A is a chromatogram of the separation of the enantiomers of verapamil and was obtained using an electrolyte containing 0.1% (w/v) succinylated-β-cyclodextrin and 25 mM bis-tris propane.

FIG. 15B is a chromatogram of the attempted separation of the enantiomers of verapamil and was obtained using an electrolyte containing 0.1% (w/v) succinylated-β-cyclodextrin and 25 mM sodium tetraborate.

FIG. 16A is a chromatogram of the separation of the enantiomers of methylbenzylamine, resolution of 1.5 was obtained using an electrolyte containing 5 mM 18-crown-6-tetracarboxylic acid and 25 mM bis-tris propane.

FIG. 16B is a chromatogram of the attempted separation of the enantiomers of methylbenzylamine, and was obtained using an electrolyte containing 5 mM 18-crown-6-tetracarboxylic acid and 25 mM sodium tetraborate.

FIG. 17 is a chromatogram showing the baseline separation of the diastereomers of nadolol using an electrolyte containing 25 mM lauric acid and 50 mM bis-tris propane, pH adjusted to 8.5 with phosphoric acid.

FIG. 18 is a chromatogram that shows no separation for the diastereomers of nadolol, and was obtained with an electrolyte containing 25 mM lauric acid, 25 mM disodium phosphate/25 mM sodium tetraborate, pH 8.5 with phosphoric acid.

FIG. 19 is a chromatogram showing the separation of eleven priority pollutant phenols using an electrolyte containing 43 mM protonated dodecylsulfate and 43 mM bis-tris propane, pH 8.6 (unadjusted).

FIG. 20 is a chromatogram showing the separation of the same sample using an electrolyte containing 43 mM sodium dodecylsulfate and 21.5 mM disodium phosphate/21.5 mM sodium tetraborate, pH adjusted to 8.6 with phosphoric acid.

Detailed Description of the Invention

In the present invention, electrolytes are prepared with anionic EKC additives in the protonated form (as opposed to the conjugate base salt form) and free polyamino (as opposed to the conjugate acid salt form) compounds as the buffering agent. These EKC buffer/additive combinations are characterized by low conductivity, since there are no highly mobile inorganic ions present. Furthermore, the electroosmotic flow is low since these free polyamines greatly reduce it. Surprisingly, separations can be performed at higher voltages in larger internal diameter capillary columns than are typically used in EKC. Higher resolution is obtained because of the higher voltages and lower electroosmotic flow, *and* greatly improved detectability is realized because of the capillary column's larger internal diameter.

Figure 1 is a structural formula depiction of a preferred embodiment of the invention, the anionic chiral surfactant additive class which was used in some of the separations which follow, specifically (S)-N-dodecoxycarbonylyvaline, described further in US Pat. App. Ser. No. 08/124,681, filed Sept. 20, 1993, and PCT/US94/10655, filed Sept. 20, 1994, both of which are incorporated herein by reference. This surfactant contains a carboxylate head group and is fully anionic at pHs above 6.5. The surfactant is used in its protonated form, and is available from Waters Corporation, 34 Maple St., Milford, MA 01757. A short list of other chiral surfactants disclosed in both applications cited immediately above includes, but is not limited to: (S)-2-[(1-oxododecoxy)amino]-3-methyl-1-sulfooxybutane; (R)-N-dodecoxycarbonylvaline; (S)-N-dodecoxycarbonyl-tert-leucine; (S)-N-tetradecoxycarbonylvaline; and (S)-N-dodecoxycarbonylphenylglycine; (S)-N-dodecoxycarbonylserine, (S)-N-dodecoxycarbonylalanine, (S)-N-dodecoxycarbonylleucine, and (S)-N-dodecoxycarbonylproline.

However, the electrolyte combination of protonated additive and buffer is not limited to chiral surfactants as additives, but may also be used in combination with other protonated EKC additives such as carboxylic and sulfonic acid derivatives of: cyclodextrin; macrocyclic antibiotics (e.g. vancomycin); achiral and chiral synthetic surfactants (examples given above); achiral and chiral polymers; and biomolecules (e.g. amino acids, peptides, proteins, oligosaccharides, nucleic acids, oligonucleotides, etc.).

In examples 8-11, polyamines other than bis-tris propane (structures given in Figure 8) were used in combination with (S)-N-dodecoxycarbonylvaline to resolve the enantiomers of several chiral compounds with low EOF and wattage. These examples illustrate that electrolytes with low EOF and wattage can be obtained with (S)-N-dodecoxycarbonylvaline in combination with a variety of polyamines, i.e., the invention is not limited to bis-tris propane. It should be apparent to those skilled in the art that improved detectability would be obtained when using the surfactant with these polyamines vs. inorganic buffers.

Specifically with reference to the additive/polyamine buffer combination of a diamine and a chiral surfactant, their structures are disclosed in Figures 1 and 5. Fig. 1 is the structural formula of the protonated chiral surfactant (S)-N-dodecoxycarbonylvaline. In solution the chiral surfactant will form micelles above its

critical micellar concentration (cmc), the outer periphery of the micelle presenting the anionic portion of the head group to the aqueous environment. The charged anionic head group of the surfactant will give up its proton to a more basic amine, and so will be present in its charged form. In a preferred embodiment, the polyamine used herein is bis-tris propane (Fig. 5), a diamine buffer that is available as the free amine. The pKas of the two amine moieties of the diamine are 6.8 and 9.0, respectively. It has good buffering capacity over the pH range of 6.3-9.5. When dissolved in water, bis-tris propane causes the pH to increase since it is a base. The additive surfactant, which is in the acid form, donates its proton to the solution, causing the pH of the solution to decrease. It is hypothesized that a complex forms comprising the negatively-charged anionic surfactant and the positively charged polyamine. By choosing the proper concentration ratio of bis-tris propane to (S)-N-dodecoxycarbonylvaline, which is well within the skill of one of ordinary skill in the art, an electrolyte pH which is within the buffering range of bis-tris propane can be achieved. The conductivity of the resulting electrolyte is lower than that afforded by electrolytes prepared with inorganic buffers, because there is no contribution from the inorganic counterions prevalent in salts, such as sodium or chloride. It was surprising to find that the electrolyte used in the chromatograms of Figures 3 and 4, despite the adjustment of pH with phosphoric acid, could be used at high field strength in 100 μm capillaries with minimal joule heat generated.

Another benefit of bis-tris propane when used with protonated additives in EKC is that the electroosmotic flow ("EOF") is reduced, and it has been shown previously that diamino compounds alone reduce the electroosmotic flow in untreated fused silica capillaries (Landers, J.P., Oda, R.P., Madden, B.J. and Spelsberg, T.C. Analytical Biochemistry, 1992, (205) 115-124; Song, L., Ou, Q. and Yu, W. Journal of Chromatography, 1993, (657) 175-183). However, it is surprising that detection limits can be significantly reduced (a full order of magnitude) as a result of both the low conductivity afforded by these buffer/additive combinations, and as well as the increase in resolution due to the EOF reduction. Both factors contribute to the ability to use greater quantities of analyte for improved sensitivity. This simultaneous improvement in 1) resolution and 2) lowered conductivity results in a synergistic improvement in sensitivity, demonstrated herein.

To demonstrate that the advantages of polyamine buffers in EKC are not limited to (S)-N-dodecoxycarbonylvaline, bis-tris propane was used in combination with several other EKC additives. The resulting electrolytes were compared to ones generated with the same additive in combination with inorganic buffers, and are shown in Examples 12-16. Note that for all additives the electrolyte prepared with bis-tris propane offered superior resolution and substantially lower wattage. The additives include two anionic cyclodextrin derivatives (carboxymethylated-β-cyclodextrin and succinylated-β-cyclodextrin, examples 12-13), a chiral crown ether (18-crown-6-tetracarboxylic acid, example 14), and two achiral surfactants (lauric acid and dodecyl sulfate, examples 15-16). All of these anionic additives, with the exception of dodecyl sulfate, are commercially available in the protonated form.. Chemical structures of the additives are given in Figure 13. At the end of this section is a table summarizing the results for each additive with bis-tris propane and the inorganic buffer.

Other polyamine compounds may also come within the spirit and scope of this invention. For instance, many amines are available commercially that could be used to gain the effect demonstrated herein. Such compounds include, but are not limited to: ethylenediamine; 1,3-diaminopropane; 1,2-diaminopropane; 1,4-diaminobutane; 1,2-diamino-2-methylpropane; (+/-)-1,3-diaminopentane; 1,5-diaminopentane; 2,2-dimethyl-1,3-propanediamine; 1,6 -hexanediamine; 2-methyl-1,5-pentanediamine; 1,7-diaminoheptane; 1,8-diaminoctane; 1,9-diaminononane; 1,10-diaminodecane; 1,12-diaminododecane; n-methylethylenediamine; n-ethylethylenediamine; n-propylethylenediamine; n-isopropylethylenediamine; n,n-dimethylethylenediamine; n,n-diethylethylenediamine; n,n-diisopropylethylene diamine; n,n-dibutylethylenediamine; n,n,n-trimethylethylenediamine; n,n-dimethyl-n'-ethylethylenediamine; n,n-diethyl-n'-methylethylenediamine; n,n,n-triethylethylenediamine; n,n,n,n-tetramethylethylenediamine; n,n,n,n-tetraethylethylenediamine; n-methyl-1,3-propanediamine; n-propyl-1,3-propanediamine; n-isopropyl-1-3-propanediamine; 3-dimethylaminopropylamine; 3-diethylaminopropylamine; 3-(dibutylamino)propylamine; n,n'-dimethyl-1,3-propanediamine; n,n'-diethyl-1,3-propanediamine; n,n'-diisopropyl-1,2-propanediamine; n,n,n'-trimethyl-1,3-propanediamine; n,n,n,'n'-tretramethyl-1,3-propanediamine; n,n,n,'n'-tetraethyl-1,3-propanediamine; n,n,n,'n'-tetramethyl-1,3-butanediamine; n,n, 2,2-tetramethyl-1,3-

propanediamine; n,n,n,'n'-tetramethyl-1,4-butanediamine; 2-amino-5-diethylaminopentane; n,n'-dimethyl-1-6-hexanediamine; n,n,n,'n'-tetramethyl-1,6-hexanediamine; tris(dimethylamino)methane; diethylenetriamine; n,n,n,'n,'n'-pentamethyldiethylenetriamine; n-(2-aminoethyl)-1,3-propanediamine; 3,3'-diamino-n-methyldipropylamine; 3,3'-iminobispropylamine; 3,3'-iminobis(n,n-dimethylpropylamine); spermidine; bis(hexamethylene)triamine; triethylenetetramine; 1,1,4,7,10,10-hexamethyltriethylenetetramine; n,n'-bis(3-aminopropyl)-ethylenediamine; n,n'-bis(2-aminoethyl)-1,3-propanediamine; n,n'-bis(3-aminopropyl)-1,3-propanediamine; spermine; tris(2-aminoethyl)amine; tetraethylenepentamine; pentaethylenehexamine; 5-amino-2,2,4-trimethyl-1-cyclopentanemethylamine mixture of isomers; 4,4'-methylenebis(cyclohexylamine); 4,4'-methylenebis(2-methylcyclohexylamine); 1,2-diaminocyclohexane; cis-1,2-diaminocyclohexane; (+/-) trans-1-2-diaminocyclohexane; (1S,2S)-(+)1,2-diaminocyclohexane; (1R,2R)-(-)-1,2-diaminocyclohexane; trans-1,4-diaminocyclohexane; 1,3-cyclohexanebis-(methylamine); n-cyclohexyl-1-3-propanediamine; 1,8-diamino-p-menthane; n,n'-diethyl-2-butene-1,4-diamine; n,n,n',n'-tetramethyl-2-butene-1,4-diamine; tetrakis(dimethylamino)-ethylene; 2,2'-oxybis(ethylamine); 4,9-dioxa-1,12-dodecanediamine; 4,7,10-trioxa-1, 13-tridecanediamine; 1,3-diamino-2-hydroxypropane; 2-(2-aminoethylamino)ethanol;1,3-bis(dimethylamino)-2-propanol; n,n'-bis(2-hydroxyethyl)ethylenediamine; n,n,n,'n'-tetrakis(2-hydroxy-propyl)ethylenediamine; pentrol; 2-(aminomethyl)-1-ethylpyrrolidine; 2-(2-aminoethyl)-1-methylpyrrolidine; (S)-(+)-1,(2-pyrrolidinylmethyl)pyrrolidine; dipiperidinomethane; 1,1'-methylenebis(3-methylpiperidine); 4-piperidinopiperidine; 4,4'-ethylenedipiperidine 4.4'-trimethylenedipiperidine; 4,4'-trimethylenebis(1-methylpiperidine); 4,4'-trimethylenebis(1-piperidineethanol); 1-(2-aminoethyl)-piperidine; 1-(3-aminopropyl)-2-pipecoline; 1-methyl-4-(methylamino) piperidine; 4-(aminomethyl) piperidine; 4-amino-2,2,6,6-tetramethylpiperidine; 4-dimethylamino-2-2,6,6-tetramethylpiperidine; 2-methyl-2-imidazoline; 4,4-dimethyl-2-imidazoline; 2-methylthio-2-imidazoline; piperazine; 1-methylpiperazine; 1,4-dimethylpiperazine; 2-methylpiperazine; 2,6-dimethylpiperazine; trans-2,5-dimethylpiperazine; 4-(dimethylamino)-1,2,2,6,6-pentamethylpiperidine; 1-(3-chloropropyl)piperazine; 1-(2-hydroxyethyl)piperazine; 1,4-bis(2-hydroxyethyl)piperazine; 1-(2-(2-

hydroxyethoxy)ethyl) piperazine; 1-amino-4-methylpiperazine; 1-amino-4-(2-hydroxyethyl)piperazine; 1-(2aminoethyl)piperazine; 1,4-bis(3-aminopropyl)-piperazine hexetidine; mixture of stereoisomers, 1,4,5,6-tetrahydropyrimidine; homopiperazine; 1,3,5-trimethylhexahydro-1,3,5-trizaine; 1,3,5-triethylhexahydro-1,3,5-triazine; 1,4,7-triazacyclononane; 1,4,7-trimethyl-1,4,7-trizacyclononane; 1,5,9-triazacyclododecane; 1,5,9-trimethyl-1,5,9-triazacyclododododecane; cyclen; 1,4,8,11-tetraazacyclotetradecane; 1,4,8,11-tetramethyl-1,4,11-tetraazacyclotetradecane; 1,4,8,12-tetraazacyclopentadecane; hexacyclen trisulfate; 1,4,7,10,13,16-hexamethyl-1,4,7,10,13,16-hexaazacyclooctadecane; 1,4-diazabicyclo(2.2.2)octane; Dabco 33-LV; (-)sparteine sulfate pentahydrate; 1,3,4,6,7,8-hexahydro-2h-pyrimido(1,2-a)pyrimidine; 1,3,4,6,7,8-hexahydro-1-methyl-2H-pyrmidido(1,2-A)-pyrimidine; hexamethylenetetramine; 4-(2-aminoethyl)morpholine; 4-(2-dimethylamino)ethyl)morpholine; 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane; 4,7,13,18-tetraoxa-1,10-diazabicyclo(8,5,5)eicosane; 4,7,13,16,21,22-pentaoxa-1,10-diazabicyclo(8,8,5)-tricosane; and 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo(8,8,8)-hexacosane.

Having now generally described this invention, the same will become better understood by reference to certain specific examples which are included herein for purposes of illustration only and are not intended to be limiting unless otherwise specified. All US patents and patent applications cited herein are fully incorporated by reference.

Examples

Experimental Apparatus and Conditions for Examples: Capillary electrophoretic separations were performed with a Waters Quanta® 4000E CE unit, Waters Corporation, Milford, MA. Separations were performed in fused silica capillaries, the dimensions specified in the specific examples. Likewise, the magnitude of the voltages are also described in the specific examples. Injection was achieved by raising the inlet end of the capillary immersed in the sample solution to a height of 10 cm above the outlet end for either 2 or 5 seconds. On-column UV detection was performed at 214 nm. Data collection was achieved with Waters Millennium® software v.2.1(Waters Corporation, Milford, MA).

Example 1. Separation of 99% (+) and 1% (-) Ephedrines in normal buffer, 2-second injection. Figure 2 shows the separation of 1% (-)-ephedrine in the presence of (+)-ephedrine with 25 mM (S)-N-dodecoxycarbonylvaline and common MEKC buffering agents, 25 mM Na_2HPO_4/25 mM $Na_2B_4O_7$. The pH of 25 mM Na_2HPO_4/25 mM $Na_2B_4O_7$ is 9.5 in the absence of the surfactant. However, because the surfactant is in the protonated form, when it is dissolved in the electrolyte, the pH drops to 8.8. The electrolyte pH is then adjusted to 8.5 with 1.4 M phosphoric acid. The separation was performed in a 60 cm x 100 μm i.d. capillary with a voltage of +15,000 volts. The current was 275 μA. The sample was 0.01 mg/mL (-)-ephedrine and 0.99 mg/mL (+)-ephedrine in water.

In the top separation, a hydrostatic injection time of 2 seconds was employed. The resolution was 1.3. The signal for the minor peak was 0.5 mAU, while the noise, measured as peak-to-peak from 9.0 to 9.5 minutes, was 0.25 mAU. Therefore, the S/N was 2, which is generally defined as the detection limit.

Example 2. Separation of 99% (+) and 1% (-) Ephedrines in normal buffer, 5-second injection. To increase detectability, a 5 second hydrostatic injection time was employed. This separation is shown in the bottom of Figure 2. Expectedly, resolution was not achieved at this higher sample load.

The poor resolution and high background noise (0.25 mAU) for both examples 1 and 2 with the surfactant/phosphate/borate combination is due to the fact that the power generated under these experimental conditions was 6.9 W/m. It has been recommended that power levels be kept below 1 W/m to minimize band broadening due to Joule heating (Sepaniak, M.J. and Cole, R.O. Analytical Chemistry, 1987, (59) 472-477). Therefore, a power level of 6.9 W/m would be expected to decrease resolution. Furthermore, with forced air convection and a power level of 6.9 W/m, the temperature inside the capillary has been calculated to be 35°C above the temperature outside the capillary (25°C in this case) (Nelson, R.J. et al. *supra*). At a temperature of 60 °C, the electrolyte may outgas, evaporate, and/or degrade, which would explain the high noise level. Furthermore, reproducibility would suffer due to electrolyte evaporation and degradation.

Example 3: Separation of 99% (+) and 1% (-) Ephedrines with bis-tris propane buffer, 2-second injection. Figure 3 shows the separation of 1% (-)-

ephedrine in the presence of (+)-ephedrine with 25 mM (S)-N-dodecoxycarbonylvaline and 50 mM bis-tris propane (see Fig. 5), a biological buffer available from Aldrich Chemical Co. Inc., 1001 W. St. Paul Ave., Milwaukee, WI 53233. In the absence of surfactant, the pH of 50 mM bis-tris propane is 10.6. When the electrolyte is made 25 mM in the surfactant, the pH drops to 9.3. The electrolyte pH was then adjusted to 8.5 with 1.4 M phosphoric acid. The separation was performed in a 60 cm x 100 μm i.d. capillary with a voltage of +15,000 volts. The current was 35 μA.

The top separation in Figure 3 employed a 2 second hydrostatic injection. The resolution was 3.5. The signal for the minor peak was 0.5 mAU, while the noise, measured as peak-to-peak from 19.0 to 19.5 minutes, was 0.07 mAU. The S/N was 7.1, 3X the S/N obtained with the phosphate/borate buffer (Figure 2, top separation). The improved S/N is due to the decreased noise with the bis-tris propane buffer (0.07 mAu vs. 0.25 mAU). The power level with the bis-tris propane buffer was 0.9 W/m, below the recommended maximum of 1 W/m. Furthermore, the temperature rise above ambient was calculated to be 4.5 °C at a power level of 0.9 W/m with forced air convection (Nelson, R.J. *supra*). The lower power level affords higher resolution and better detectability.

Example 4. Separation of 99% (+) and 1% (-) Ephedrines in bis-tris propane buffer, 5-second injection. The bottom separation in Figure 3 employed a 5 second hydrostatic injection. Note that sufficient resolution (Rs = 1.4) is maintained while the S/N increases to 18.6. The higher resolution obtained with the surfactant/bis-tris propane combination allows more sample to be injected for better detectability while still maintaining sufficient resolution.

Example 5. Separation of 99.9% (+) and 0.1% (-) Ephedrines in bis-tris propane buffer, 5-second injection. This point is further illustrated in Figure 4, where a 0.1% level of (-)-ephedrine can be detected with a 5 second hydrostatic injection. In this case, the S/N was 2.2. Due to a lower noise level and higher resolution, the surfactant/bis-tris propane combination offers an order of magnitude decrease in detection limit compared to the one buffered with an equivalent concentration of phosphate/borate (0.1% vs. 1.0%).

Examples 6-7. Separation of racemic (+/-) Norephedrines in both buffers. Figures 6A and 6B show the separation of norephedrine enantiomers using 25 mM (S)-

N-dodecoxycarbonylvaline and 25 mM Na_2HPO_4/25 mM $Na_2B_4O_7$ (Fig. 6A), and the identical separation using 50 mM bis-tris propane buffer (Fig. 6B), both at pH 8.5. The voltage was 15 kV, the capillary 50 μm i.d. x 60 cm, the hydrostatic injection time 2 seconds, and the sample 0.4 mg/mL racemic norephedrine dissolved in electrolyte.

With phosphate/borate (Fig. 6A), the current was 45 μA, resulting in a power level of 1.1 W/m. The resolution was 3.0 and the electroosmotic mobility was 5.7×10^{-4} cm^2/Vs. With bis-tris propane (Fig. 6B), the current was 10.5 μA, resulting in a power level of 0.3 W/m. The resolution was 3.9 and the electroosmotic mobility was 3.6×10^{-4} cm^2/Vs. Higher resolution was obtained with the surfactant/bis-tris propane combination due to the lower electroosmotic flow. Furthermore, because the conductivity of the surfactant/bis-tris propane combination is low, the voltage can be increased to 30 kV with the wattage only increasing to 1.1 W/m (22 μA). Analysis time is decreased from 19.8 minutes to 9.1 minutes, while the resolution actually increases to 4.0 (Figure 7). When the voltage is increased to 30 kV with the surfactant/phosphate/borate combination, the wattage increases to 7.1 W/m (142 μA) and the resolution decreases to 2.0.

Table 1 summarizes the data generated from the above examples.

TABLE 1

Separation of Ephedrine Enantiomers Using Two Surfactant/Buffer Combinations

Buffer Type	Buffer Conc. (mM)	Surfactant Conc. (mM)	Injection Time (Sec)	Ephedrine Ratio (+/-)	Power Level (w/m)	S/N (Minor Peak)	Resolution
Phosphate/ Borate	50	25	2	99/1	6.9	2	1.3
Phosphate/ Borate	50	25	5	99/1	6.9	.....	0
Bis-Tris Propane	50	25	2	99/1	0.9	7.1	3.5
Bis-Tris Propane	50	25	5	99/1	0.9	18.6	1.4
Bis-Tris Propane	50	25	5	99.9 / 0.1	0.9	2.2	1.4

The detection limit is a full order of magnitude lower for the invention (see last row,

S/N 2.2 for 0.1% ephedrine in bis-tris propane buffer, vs. top row, S/N 2.0 for 1% ephedrine in phosphate/borate buffer) than for the present state-of-the-art.

Example 8: (S)-N-Dodecoxycarbonylvaline with N,N'-bis(2-aminoethyl)-1,3-propanediamine.

The electrolyte contained 25 mM (S)-N-dodecoxycarbonylvaline and 25 mM N,N'-bis(2-aminoethyl)-1,3-propanediamine, pH 9.6 (unadjusted). The capillary was 75 μm i.d. x 35 cm length. With an applied voltage of +20 kV, the current was 27 μA, for a power level of 2 W/m. The electroosmotic flow was 1.9 x 10^{-4} cm^2/Vs, a three to four-fold reduction in the normal EOF at this pH. These conditions permitted the baseline separation of metoprolol enantiomers in 7 minutes, Figure 9.

Example 9: (S)-N-Dodecoxycarbonylvaline with pentrol.

The electrolyte contained 25 mM (S)-N-dodecoxycarbonylvaline and 50 mM pentrol, pH 8.0 with phosphoric acid. The capillary was 75 μm i.d. x 60 cm length. With an applied voltage of +30 kV, the current was 25 μA, for a power level of 1.25 W/m. The electroosmotic flow was 2.1 x 10^{-4} cm^2/Vs, a three to four-fold reduction in the normal EOF at this pH. These conditions permitted the baseline separation of benzoin enantiomers, Figure 10.

Example 10: (S)-N-Dodecoxycarbonylvaline with pentaethylenhexamine.

The electrolyte contained 25 mM (S)-N-dodecoxycarbonylvaline and 15 mM pentaethylenehexamine, pH 9.6 (unadjusted). The capillary was 75 μm i.d. x 60 cm length. With an applied voltage of +30 kV, the current was 6 μA, for a power level of 0.3 W/m. The electroosmotic flow was 3.8 x 10^{-4} cm^2/Vs, a two-fold reduction in the normal EOF at this pH. Note that this reduction was achieved with only 15 mM of this hexaaamine, and that the current generated was extremely low. These conditions permitted the baseline separation of norphenylephrine enantiomers in 6 minutes, Figure 11.

Example 11: (S)-N-Dodecoxycarbonylvaline with 4,7,10-trioxa-1,13-tridecanediamine.

The electrolyte contained 25 mM (S)-N-dodecoxycarbonylvaline and 20 mM 4,7,10-trioxa-1,13-tridecanediamine, pH 9.5 (unadjusted). The capillary was 75 μm i.d. x 60 cm length. With an applied voltage of +30 kV, the current was 10 μA, for a power level of 0.5 W/m. The electroosmotic flow was 3.4 x 10^{-4} cm^2/Vs, a two-fold

reduction in the normal EOF at this pH. Note that this reduction was achieved with only 20 mM of this diamine, and that the current generated was extremely low. These conditions permitted the baseline separation of metoprolol enantiomers, Figure 12.

Example 12: Carboxymethyl-β-cyclodextrin with bis-tris propane vs. sodium tetraborate.

The top separation (Figure 14A) was obtained using an electrolyte containing 0.1% (w/v) carboxymethyl-β-cyclodextrin and 25 mM bis-tris propane, pH adjusted to 8.5 with phosphoric acid. The capillary was 75 μm i.d. x 60 cm length. With an applied voltage of +25 kV, the current was 25 μA, for a power level of 1.0 W/m. The electroosmotic flow was 4.4×10^{-4} cm^2/Vs. The enantiomers of verapamil were separated with a resolution of 1.6. The bottom separation (Figure 14B) was obtained using an electrolyte containing 0.1% (w/v) carboxymethyl-β-cyclodextrin and 25 mM sodium tetraborate, pH adjusted to 8.5 with phosphoric acid. The capillary was 75 μm i.d. x 60 cm length. With an applied voltage of +25 kV, the current was 140 μA, for a power level of 5.8 W/m. The electroosmotic flow was 8.2×10^{-4} cm^2/Vs. The enantiomers of verapamil were separated with a resolution of only 0.2, and were not fully resolved from the EOF marker. Therefore, the electrolyte containing bis-tris propane offered an increase in resolution by a factor of eight (due to lower EOF and wattage) while decreasing the power level six-fold.

Example 13: Succinylated-β-cyclodextrin with bis-tris propane vs. sodium tetraborate.

The top separation (Figure 15A) was obtained using an electrolyte containing 0.1% (w/v) succinylated-β-cyclodextrin and 25 mM bis-tris propane, pH adjusted to 8.5 with phosphoric acid. The capillary was 75 μm i.d. x 60 cm length. With an applied voltage of +30 kV, the current was 20 μA, for a power level of 1.0 W/m. The electroosmotic flow was 4.4×10^{-4} cm^2/Vs. The enantiomers of verapamil were separated with a resolution of 3.0. The bottom separation (Figure 15B) was obtained using an electrolyte containing 0.1% (w/v) succinylated-β-cyclodextrin and 25 mM sodium tetraborate, pH adjusted to 8.5 with phosphoric acid. The capillary was 75 μm i.d. x 60 cm length. With an applied voltage of +30 kV, the current was 195 μA, for a power level of 9.8 W/m. The electroosmotic flow was 8.3×10^{-4} cm^2/Vs. The enantiomers of verapamil were separated with a resolution of only 0.4. Therefore, the electrolyte

containing bis-tris propane offered an increase in resolution by a factor of seven (due to lower EOF and wattage) while decreasing the power level ten-fold.

Example 14: 18-Crown-6-tetracarboxylic acid with bis-tris propane vs. sodium tetraborate.

The top separation (Figure 16A) was obtained using an electrolyte containing 5 mM 18-crown-6-tetracarboxylic acid and 25 mM bis-tris propane, pH adjusted to 8.5 with phosphoric acid. The capillary was 75 μm i.d. x 60 cm length. With an applied voltage of +25 kV, the current was 26 μA, for a power level of 1.1 W/m. The electroosmotic flow was 4.2×10^{-4} cm^2/Vs. The enantiomers of methylbenzylamine were separated with a resolution of 1.5. The bottom separation (Figure 16B) was obtained using an electrolyte containing 5 mM 18-crown-6-tetracarboxylic acid and 25 mM sodium tetraborate, pH adjusted to 8.5 with phosphoric acid. The capillary was 75 μm i.d. x 60 cm length. With an applied voltage of +25 kV, the current was 120 μA, for a power level of 5.0 W/m. The electroosmotic flow was 8.4×10^{-4} cm^2/Vs. The enantiomers of methylbenzylamine were not separated at all. Therefore, the electrolyte containing bis-tris propane offered baseline resolution compared to none (due to lower EOF and wattage) while decreasing the power level five-fold.

Example 15: Lauric acid with bis-tris propane vs. sodium phosphate/sodium tetraborate.

Figure 17 shows the baseline separation of the diastereomers of nadolol using an electrolyte containing 25 mM lauric acid and 50 mM bis-tris propane, pH adjusted to 8.5 with phosphoric acid. The capillary was 75 μm i.d. x 60 cm length. With an applied voltage of +25 kV, the current was 25 μA, for a power level of 1.0 W/m. The electroosmotic mobility was 3.8×10^{-4} cm^2/Vs. Figure 18 shows no separation for the diastereomers of nadolol, and was obtained with an electrolyte containing 25 mM lauric acid, 25 mM disodium phosphate/25 mM sodium tetraborate, pH 8.5 with phosphoric acid. With an applied voltage of +25 kV, the current was 270 μA, for a power level of 11 W/m. The electroosmotic mobility was 5.9×10^{-4} cm^2/Vs. In this example, bis-tris propane afford baseline resolution vs. zero resolution, and an 11-fold reduction in power level. Furthermore, S/N is improved due to lower noise and increased signal due to sharper peaks.

Example 16: Protonated Dodecylsulfate with bis-tris propane vs. Sodium Dodecylsulfate with sodium phosphate/sodium tetraborate.

Figure 19 shows the separation of eleven priority pollutant phenols using an electrolyte containing 43 mM protonated dodecylsulfate and 43 mM bis-tris propane, pH 8.6 (unadjusted). Eleven peaks can be distinguished, with all baseline resolved except the pair at 15.5 minutes. The capillary was 75 μm i.d. x 60 cm length. With an applied voltage of +25 kV, the current was 24 μA, for a power level of 1.0 W/m. The electroosmotic mobility was 4.4 x 10^{-4} cm^2/Vs. Figure 20 shows the separation of the same sample using an electrolyte containing 43 mM sodium dodecylsulfate and 21.5 mM disodium phosphate/21.5 mM sodium tetraborate, pH adjusted to 8.6 with phosphoric acid. Only eight peaks can be distinguished. The capillary was 75 μm i.d. x 60 cm length. With an applied voltage of +25 kV, the current was 200 μA, for a power level of 8.0 W/m. The electroosmotic mobility was 7.3 x 10^{-4} cm^2/Vs. In this example, the protonated version of dodecylsulfate in combination with bis-tris propane offered improved resolution (due to lower EOF and lower wattage) and an eight-fold reduction in the power level.

TABLE 2:

Summary of Different Additives with Bis-Tris Propane vs. Inorganic Buffers

	Bis-Tris Propane			Inorganic Buffer		
Additive	**EOF[1]**	**Rs**	**Power[2]**	**EOF[1]**	**Rs**	**Power[2]**
carboxymethyl-β-CD	4.4	1.6	1.0	8.2	0.2	5.8
succinylated-β-CD	4.4	3.0	1.0	8.3	0.4	9.8
18-crown-6-tetracarboxylic acid	4.2	1.5	1.1	8.4	0	5.0
lauric acid	3.8	1.5	1.0	5.9	0	11
dodecylsulfate	4.4	11 peaks	1.0	7.3	8 peaks	8.0

1- units of 10^{-4} cm^2/Vs
2- units of Watts/m

Although the foregoing invention has been described by way of illustration and

example for purposes of clarity and understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the invention, as limited only by the scope of the appended claims.

We claim:

1. A capillary electrophoresis electrolyte for separating analyte components of interest contained in a substantially aqueous sample, comprising a substantially aqueous phase, a protonated additive dispersed in the substantially aqueous phase, and a free polyamine dispersed in the substantially aqueous phase.

2. The electrolyte of claim 1 wherein the substantially aqueous phase includes an organic modifier.

3. The electrolyte of claim 1 wherein the additive comprises a protonated surfactant.

4. The electrolyte of claim 1 wherein the additive comprises a protonated chiral surfactant.

5. The electrolyte of claim 1 wherein the additive comprises a protonated achiral surfactant.

6. The electrolyte of claim 1 wherein the additive is lauric acid.

7. The electrolyte of claim 1 wherein the additive is dodecyl sulfate.

8. The electrolyte of claim 1 wherein the additive is (S)-N-dodecoxycarbonylvaline.

9. The electrolyte of claim 1 wherein the additive is a protonated cyclodextrin acid derivative.

10. The electrolyte of claim 9 wherein the additive is carboxymethyl-β-cyclodextrin.

11. The electrolyte of claim 9 wherein the additive is succinylated-β-cyclodextrin.

12. The electrolyte of claim 1 wherein the additive is a protonated crown ether acid derivative.

13. The electrolyte of claim 12 wherein the additive is 18-crown-6-tetracarboxylic acid.

14. The electrolyte of claim 1 wherein the free polyamine is a diamine.

15. The electrolyte of claim 1 wherein the diamine is bis-tris propane.

16. The electrolyte of claim 1 wherein the free polyamine is N,N'-bis(2-aminoethyl)-1,3-propanediamine.

17. The electrolyte of claim 1 wherein the free polyamine is pentrol.

18. The electrolyte of claim 1 wherein the free polyamine is pentaethylenhexamine.

19. The electrolyte of claim 1 wherein the free polyamine is 4,7,10-trioxa-1,13-tridecanediamine.

20. A method for separating analyte components of interest under capillary electrophoretic conditions contained in a substantially aqueous sample, comprising the step of contacting said sample with an effective amount of a combination of a protonated additive and a free polyamine dispersed in a substantially aqueous phase.

21. The method of claim 20 wherein the substantially aqueous phase includes an organic modifier.

22. The method of claim 20 wherein the additive is selected from the group consisting of dodecyl sulfate, (S)-N-dodecoxycarbonylvaline, carboxymethyl-β-cyclodextrin, and succinylated-β-cyclodextrin.

23. The method of claim 20 wherein the free polyamine is selected from the group consisting of bis-tris propane, N,N'-bis(2-aminoethyl)-1,3-propanediamine, pentrol, pentaethylenhexamine, and 4,7,10-trioxa-1,13-tridecanediamine.

24. A kit for separating analyte components of interest contained in a substantially aqueous sample, the kit being compartmentalized to receive in close confinement one or more containers which comprise separately or in combination a protonated additive to be dispersed in the substantially aqueous phase, and a free polyamine to be dispersed in the substantially aqueous phase.

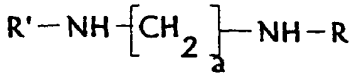
25. The kit of claim 24 wherein the substantially aqueous phase includes an organic modifier.

26. The kit of claim 24 wherein the additive comprises a protonated surfactant.

27. The kit of claim 24 wherein the additive is selected from the group consisting of dodecyl sulfate, (S)-N-dodecoxycarbonylvaline, carboxymethyl-β-cyclodextrin, and succinylated-β-cyclodextrin.

28. The kit of claim 24 wherein the free polyamine is selected from the group consisting of bis-tris propane, N,N'-bis(2-aminoethyl)-1,3-propanediamine, pentrol, pentaethylenhexamine, and 4,7,10-trioxa-1,13-tridecanediamine.

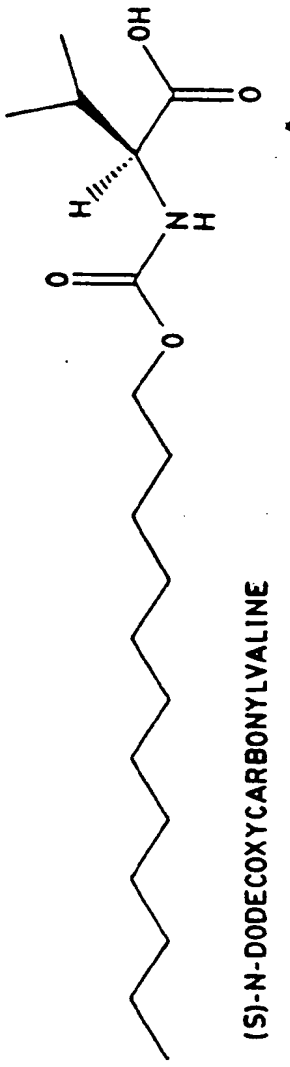
29. A capillary electrophoresis electrolyte for separating analyte components of interest contained in a substantially aqueous sample, comprising a substantially aqueous phase, a protonated additive dispersed in the substantially aqueous phase, and a free polyamine dispersed in the substantially aqueous phase, the free polyamine having the following structural formula:



wherein

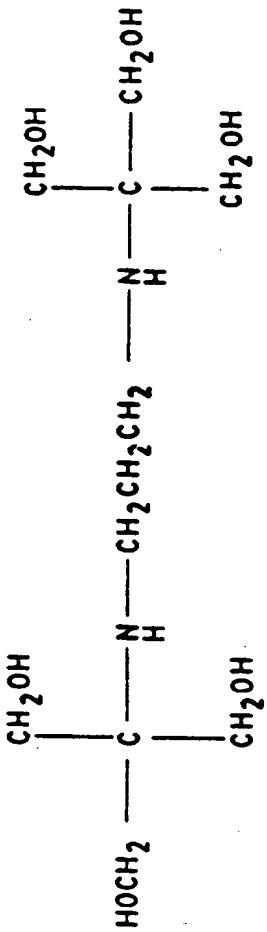
a is from about 1 to 8 methylenes; and

R and R' are the same or different and may be alkyl, alkenyly or alkynyl substituents, branched or straight chain, substituted or unsubstituted, having from about 1 to 8 carbons, and may have one or more hydrophilic moities such as hydroxy, sulfhydryl, or amine.



(S)-N-DODECOXYCARBONYLVALINE

FIG. 1



BIS-TRIS PROPANE

FIG. 5

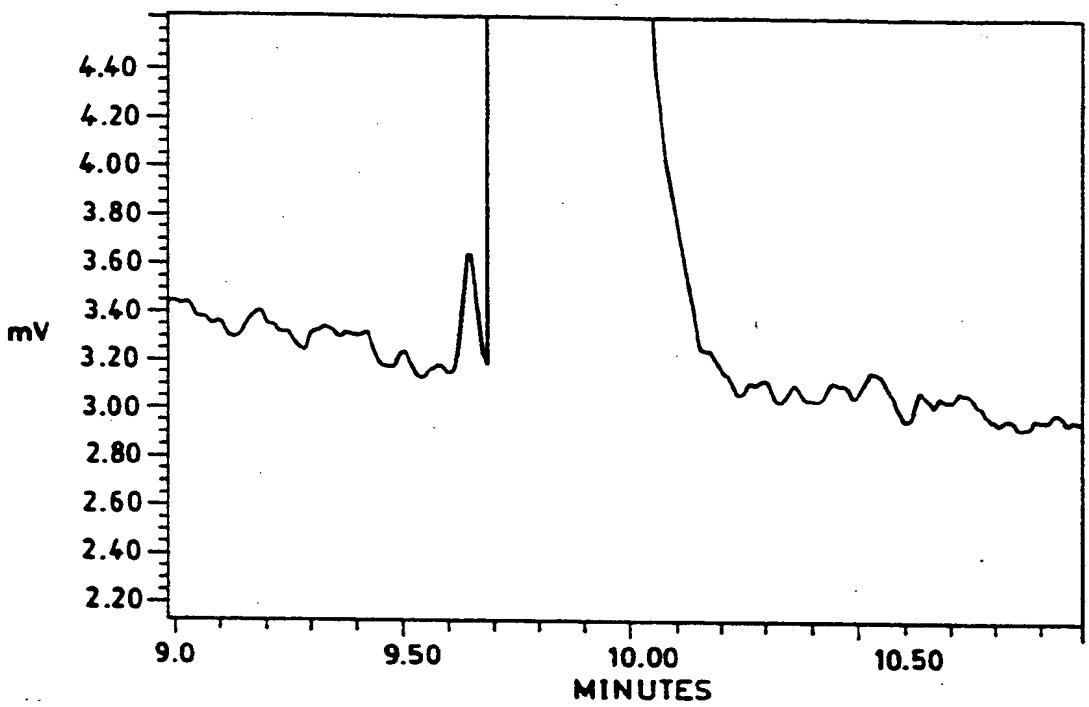


FIG. 2A

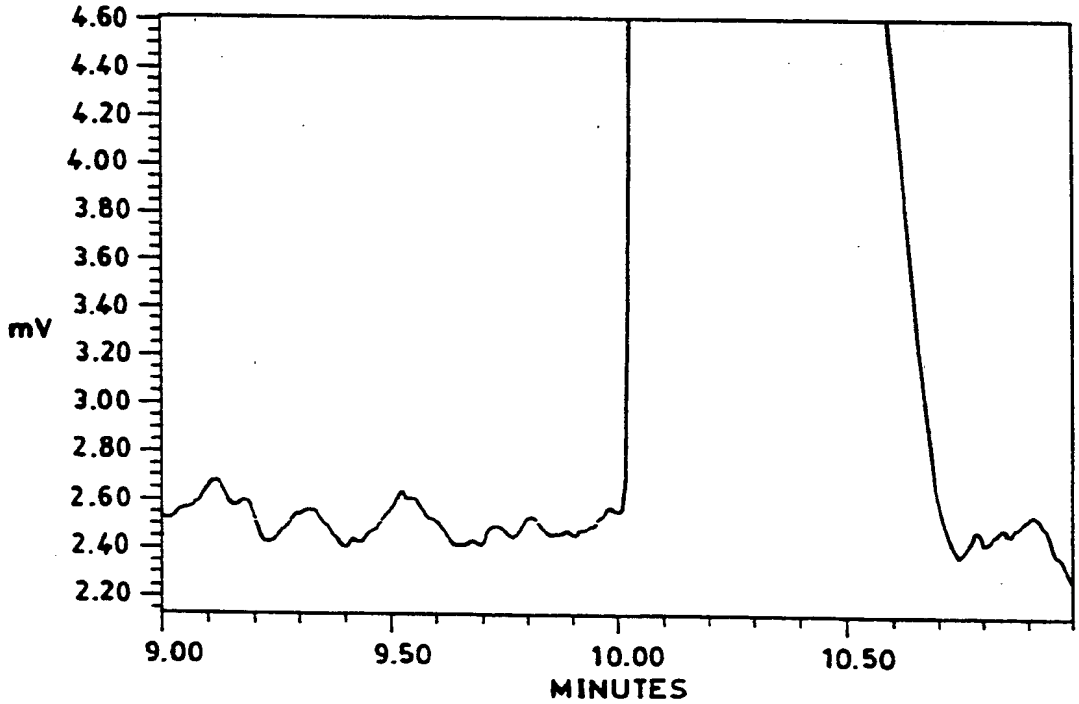


FIG. 2B

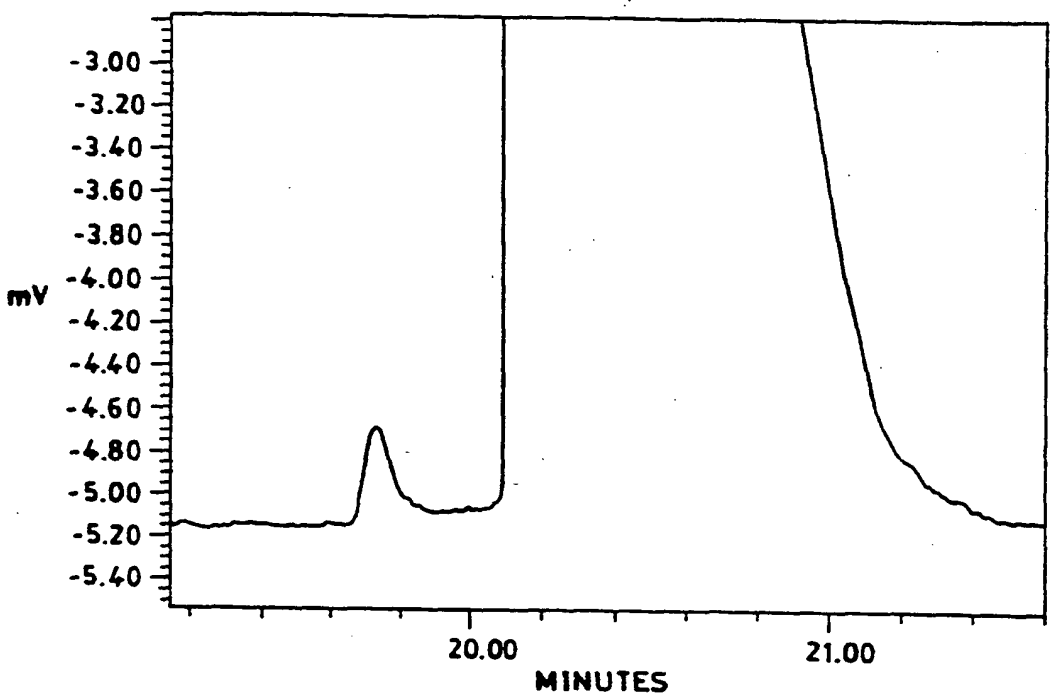


FIG.3A

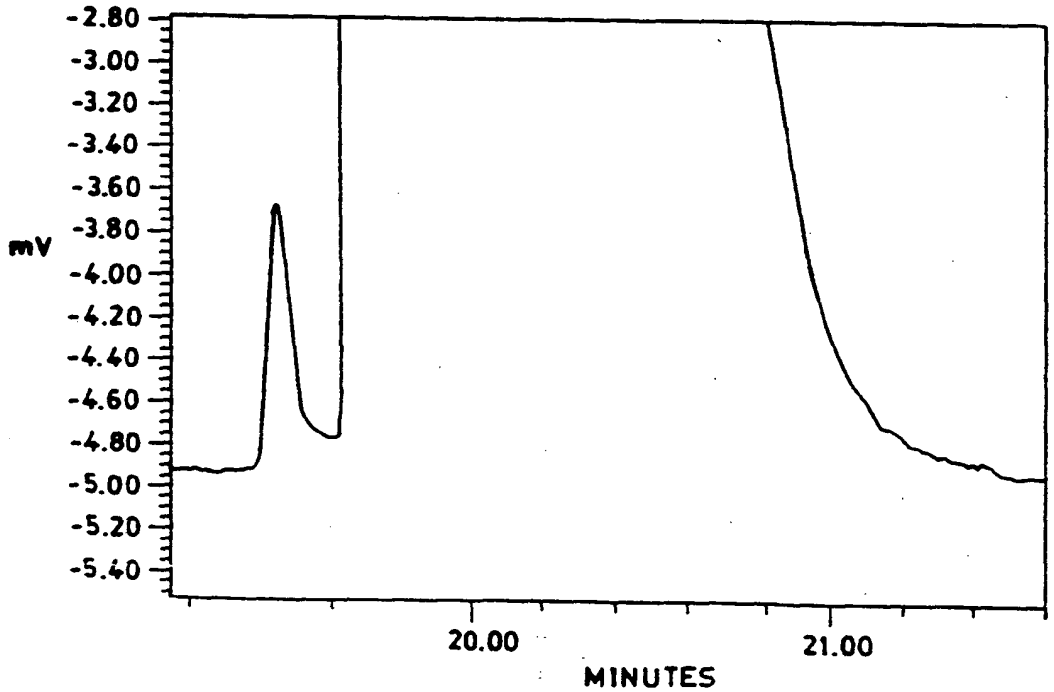


FIG. 3B

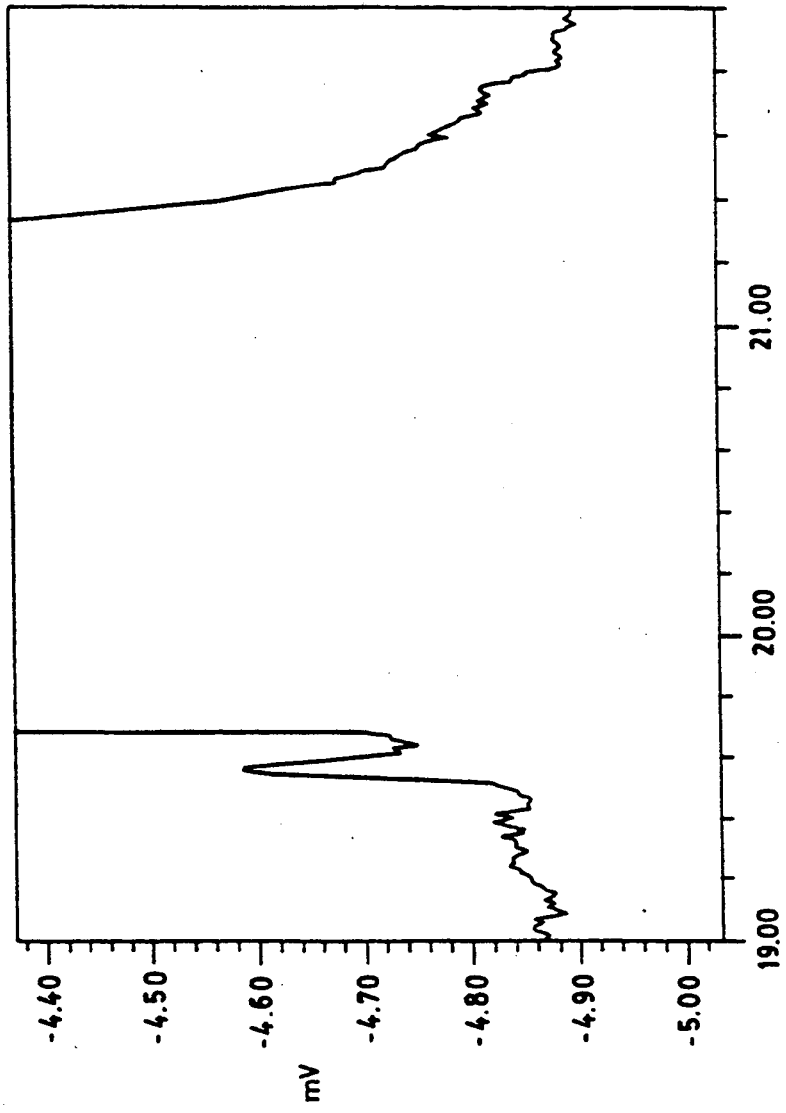


FIG. 4

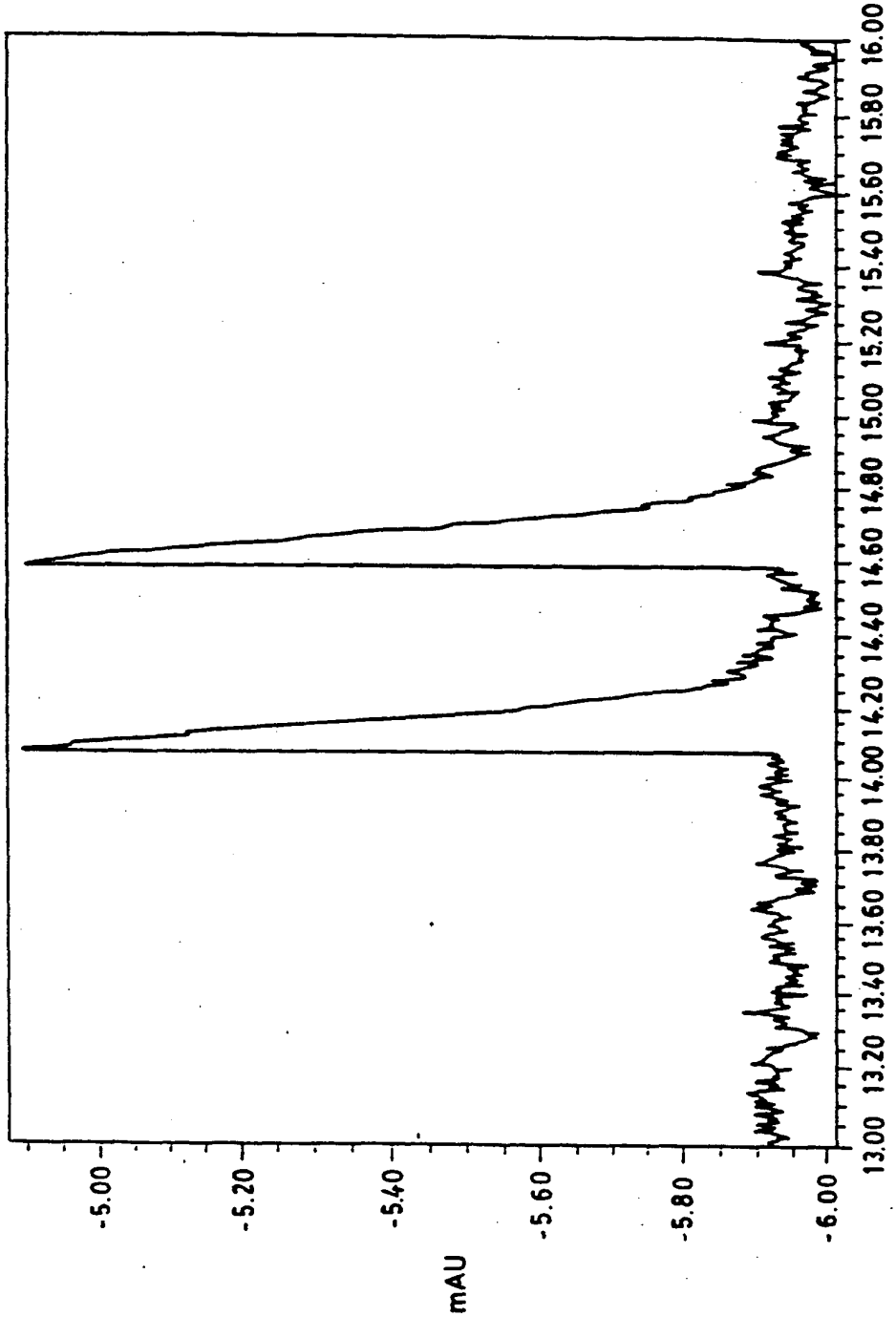


FIG. 6A

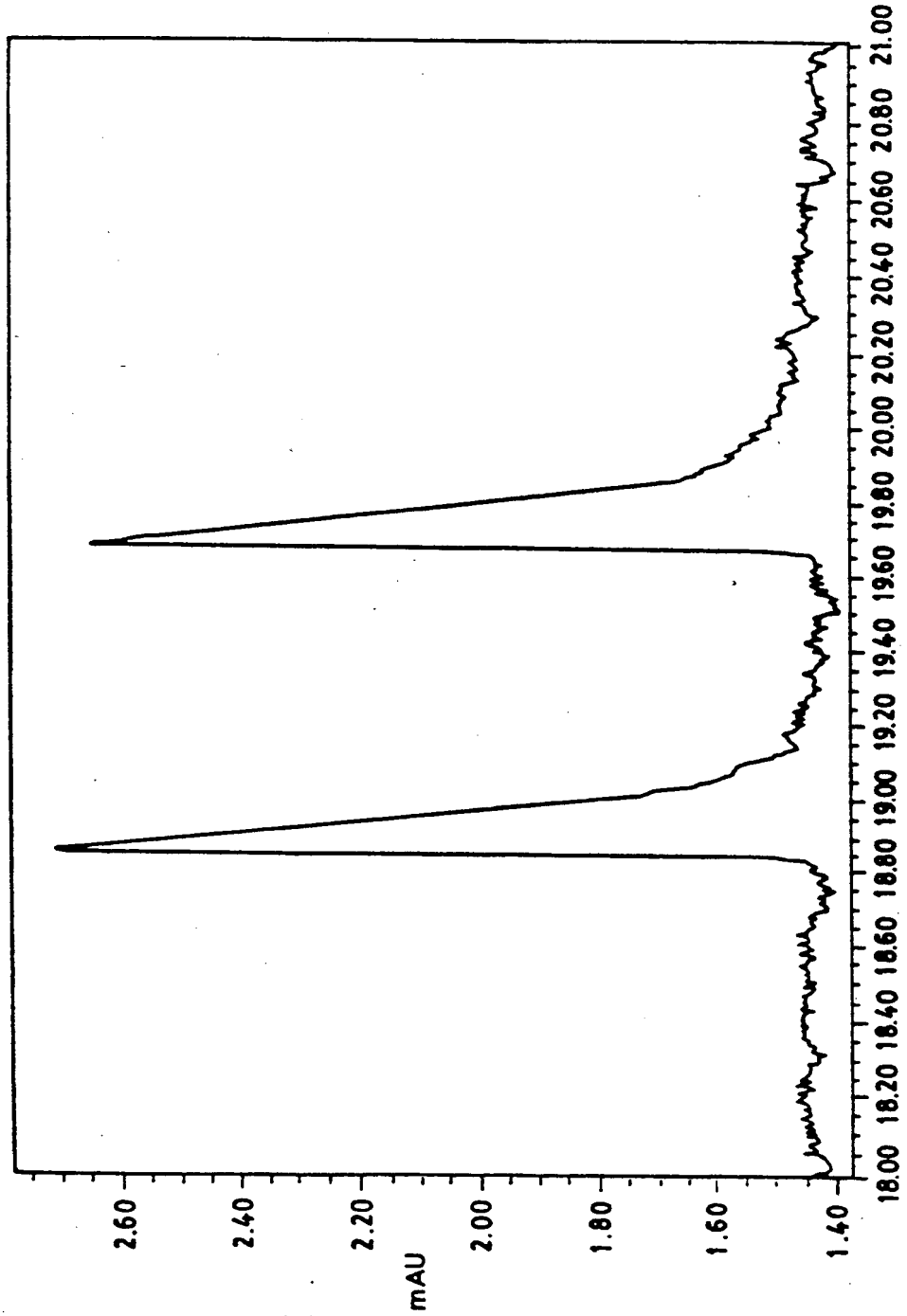


FIG. 6B

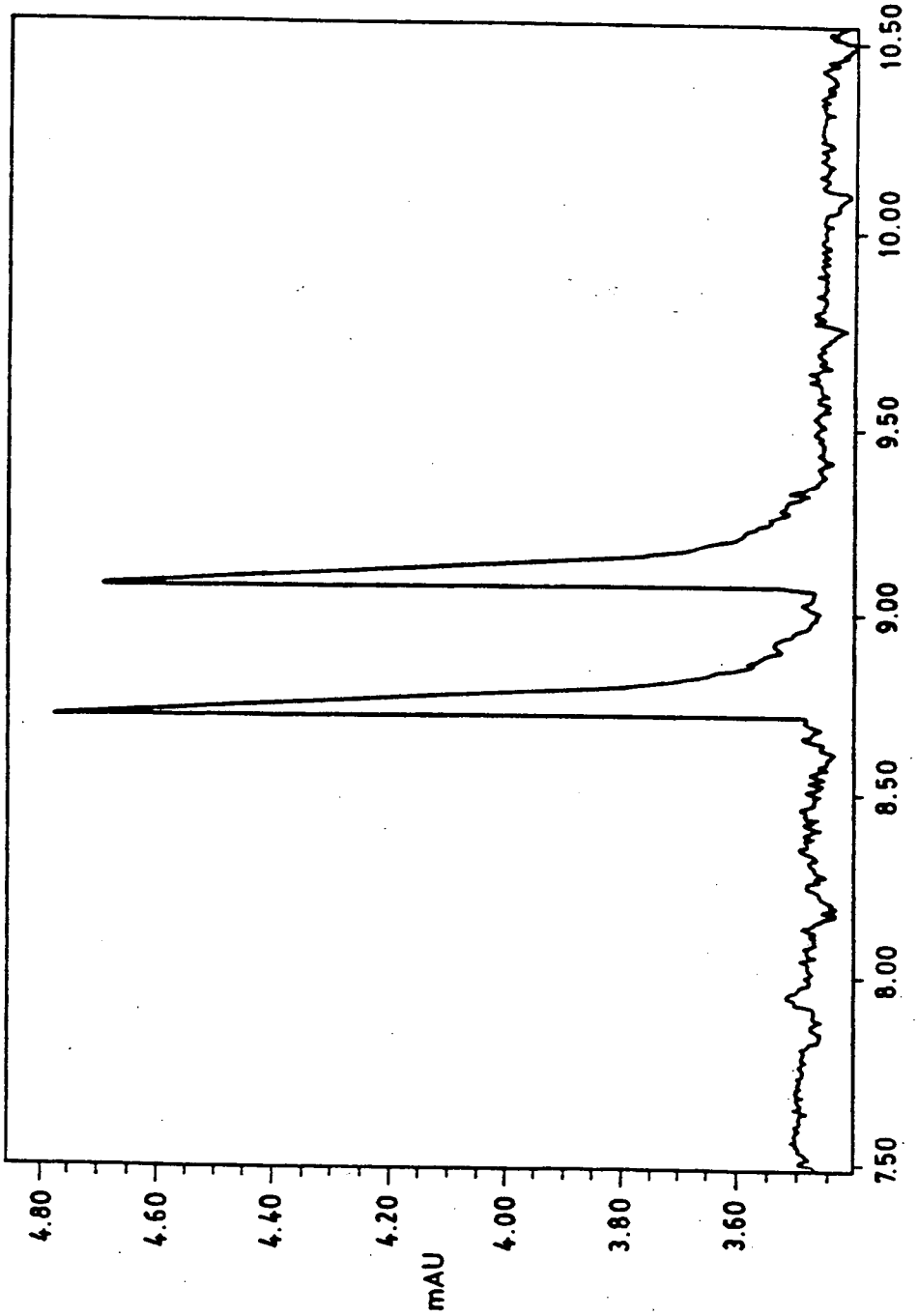


FIG.7

$NH_2CH_2CH_2NHCH_2CH_2CH_2NHCH_2CH_2NH_2$

N,N'-bis-(2-aminoethyl)-1,13-propanediamine

$CH_3CH(OH)CH_2N(CH_2CH_2N(CH_2CH(OH)CH_3)_2)_2$

pentrol

$NH_2CH_2CH_2NHCH_2CH_2NHCH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$

pentaethylenehexamine

$O(CH_2CH_2O(CH_2)_3NH_2)_2$

4,7,10-trioxa-1,13-tridecanediamine

FIG. 8

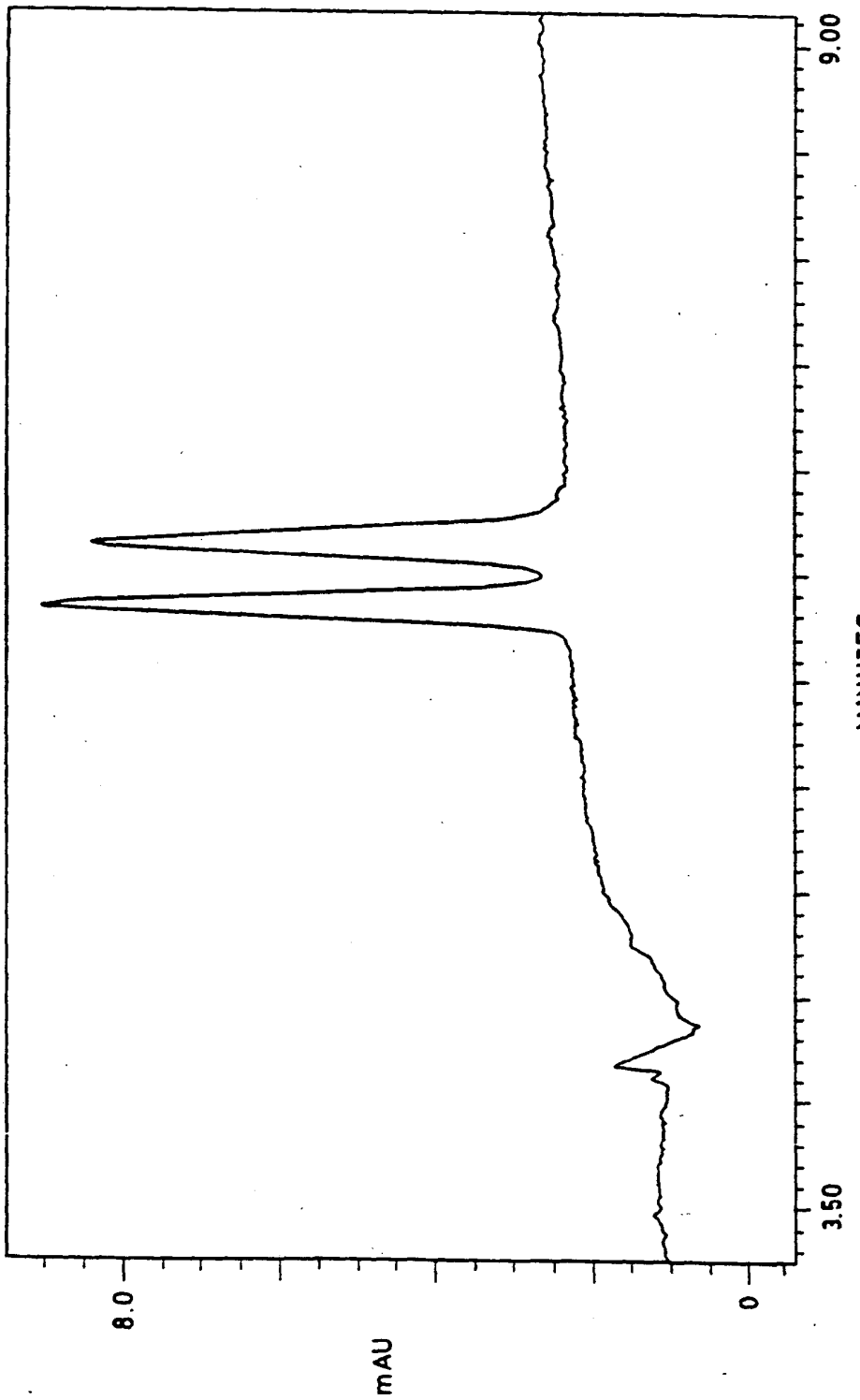


FIG. 9

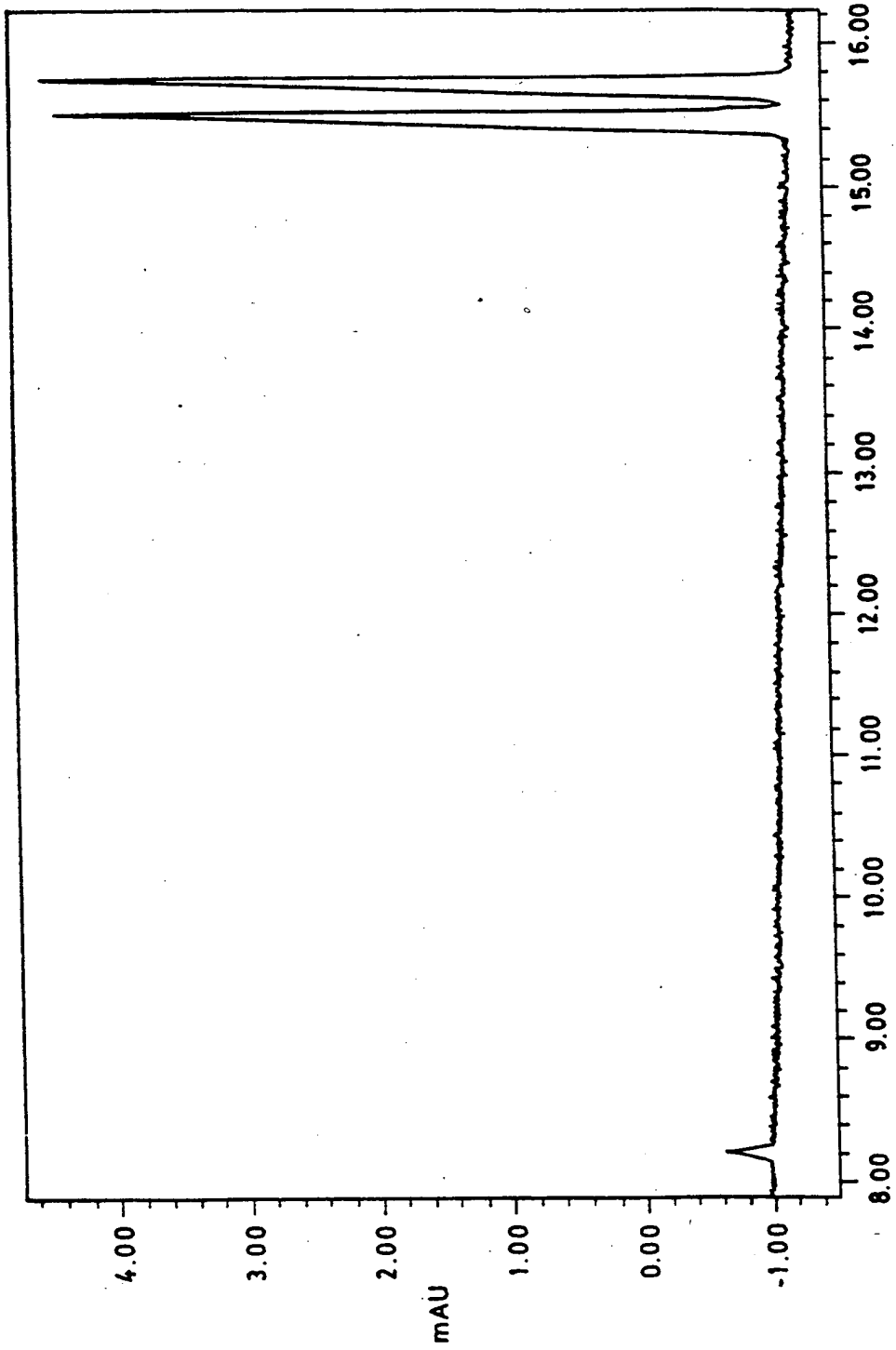


FIG. 10

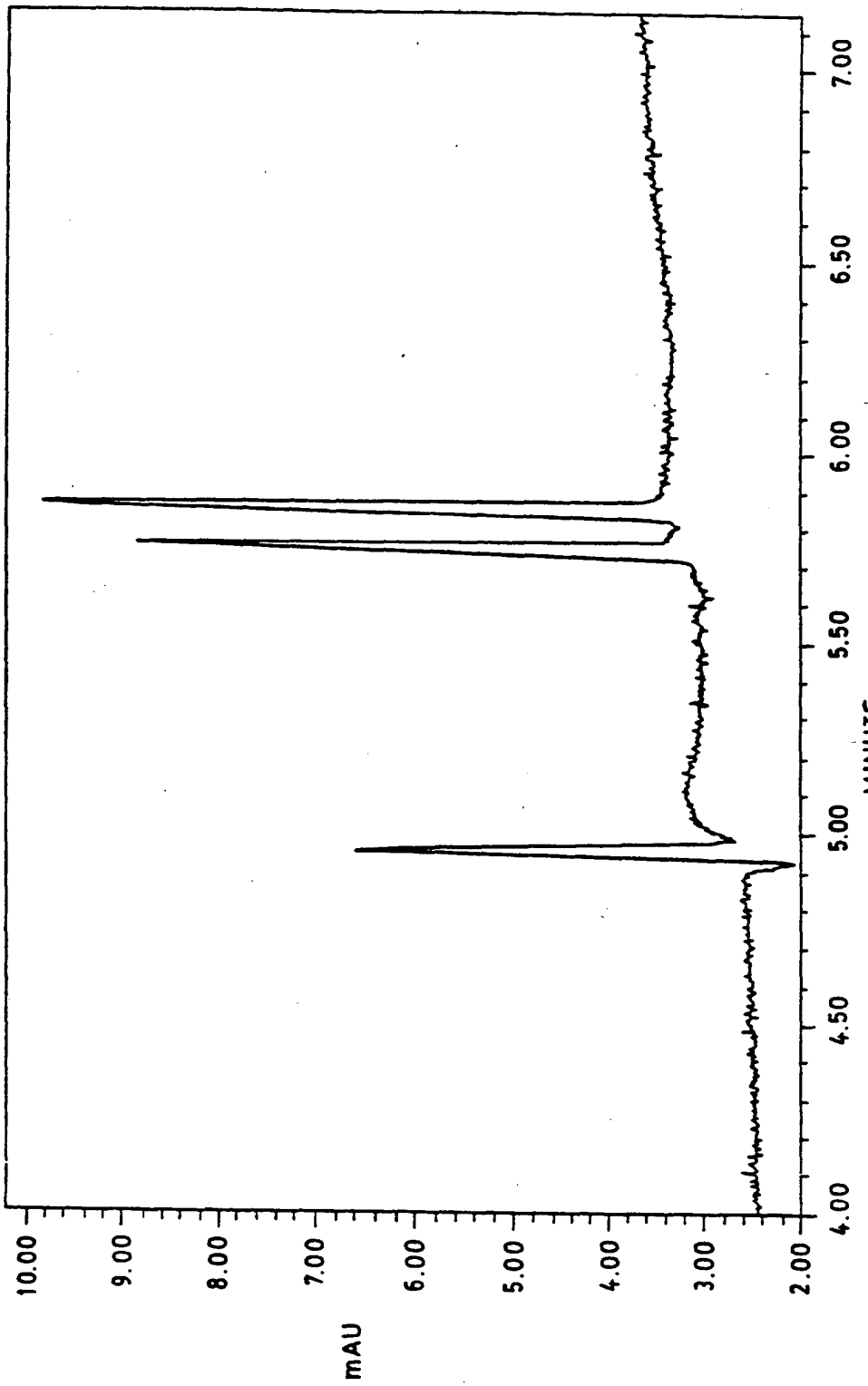


FIG. 11

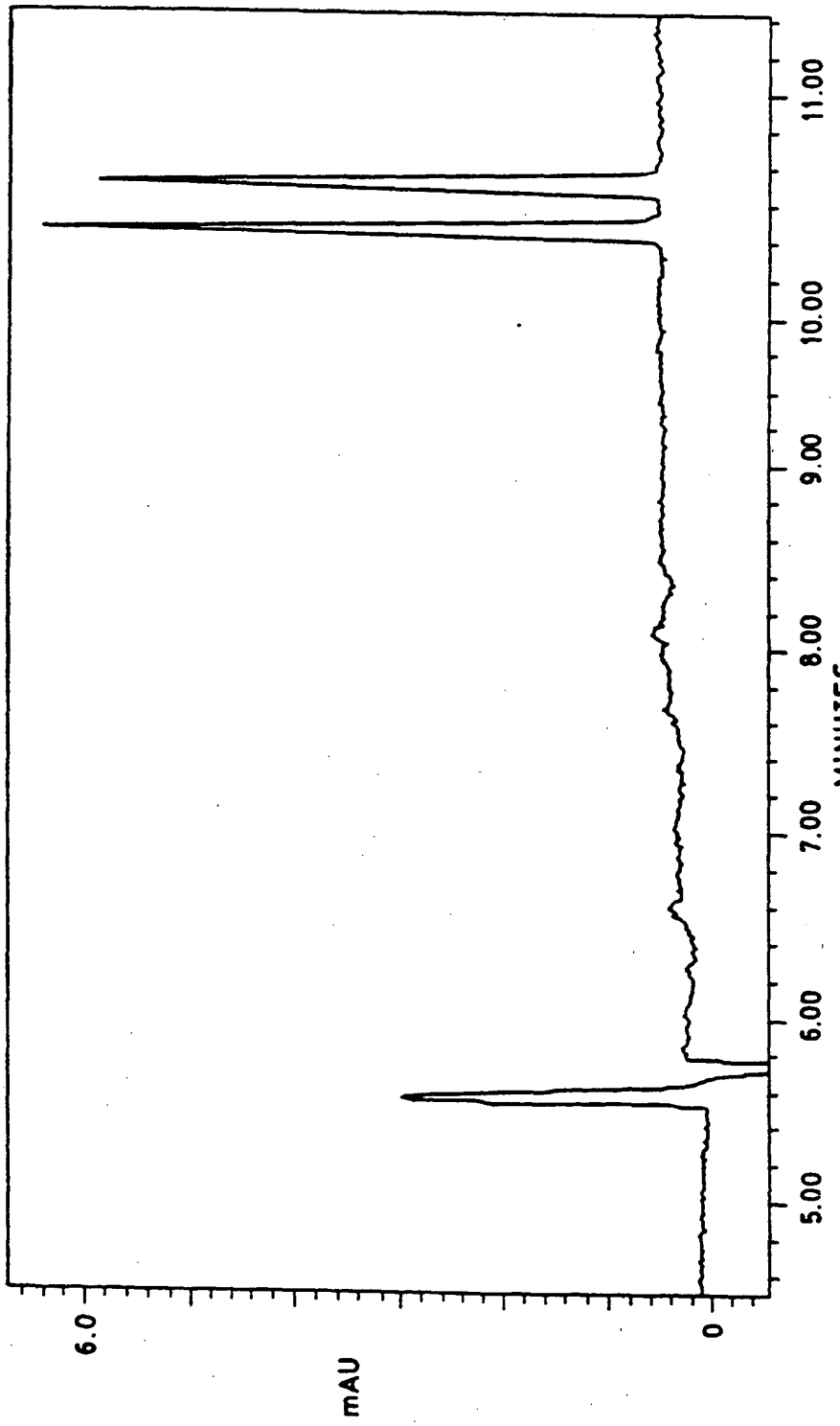


FIG. 12

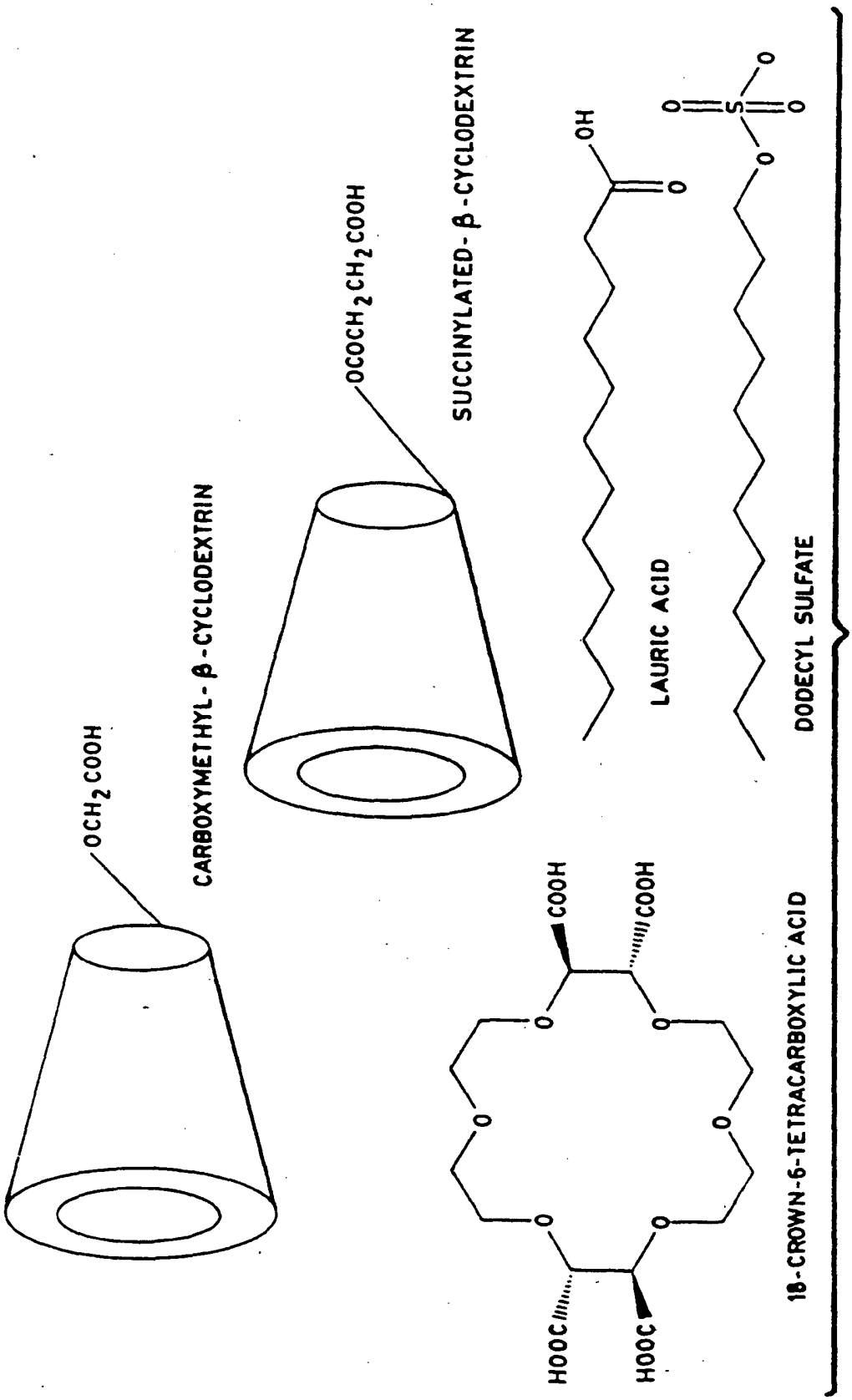


FIG. 13

FIG.14A

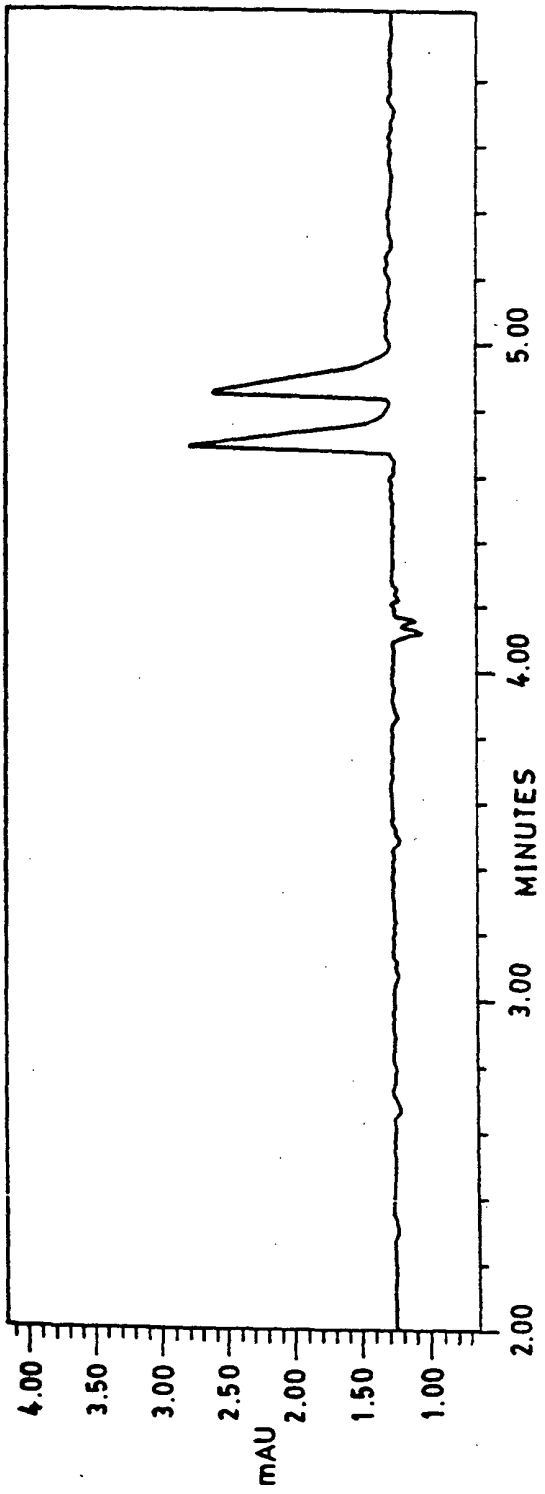


FIG.14B

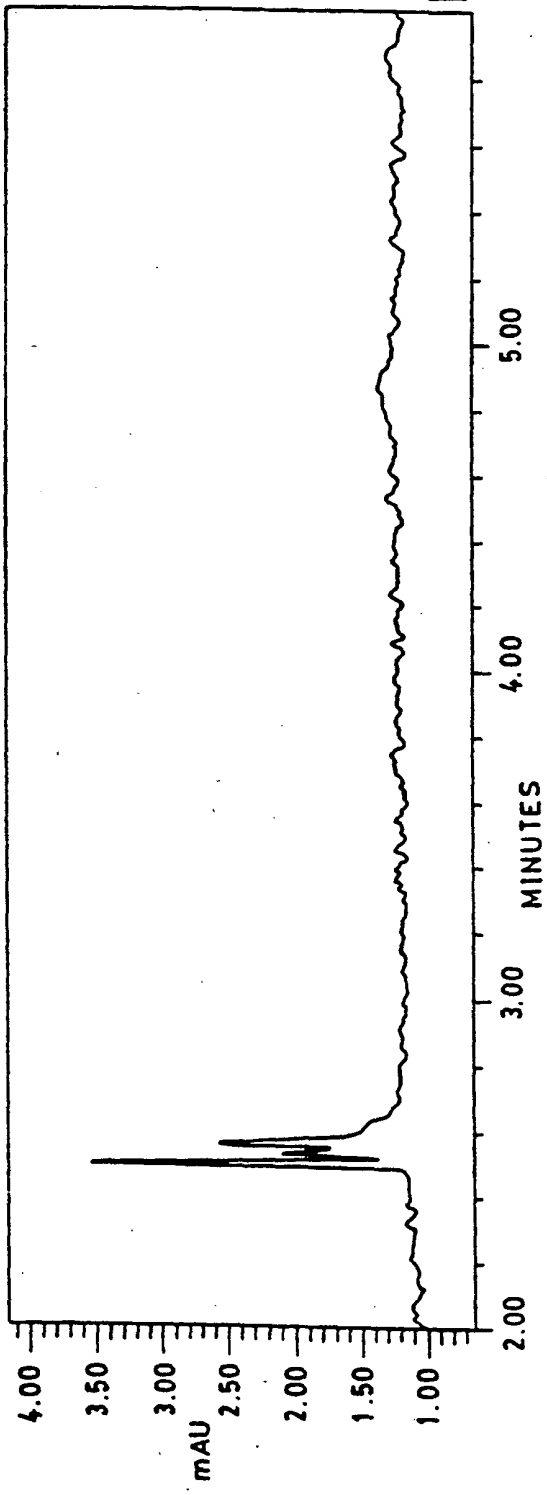


FIG.15A

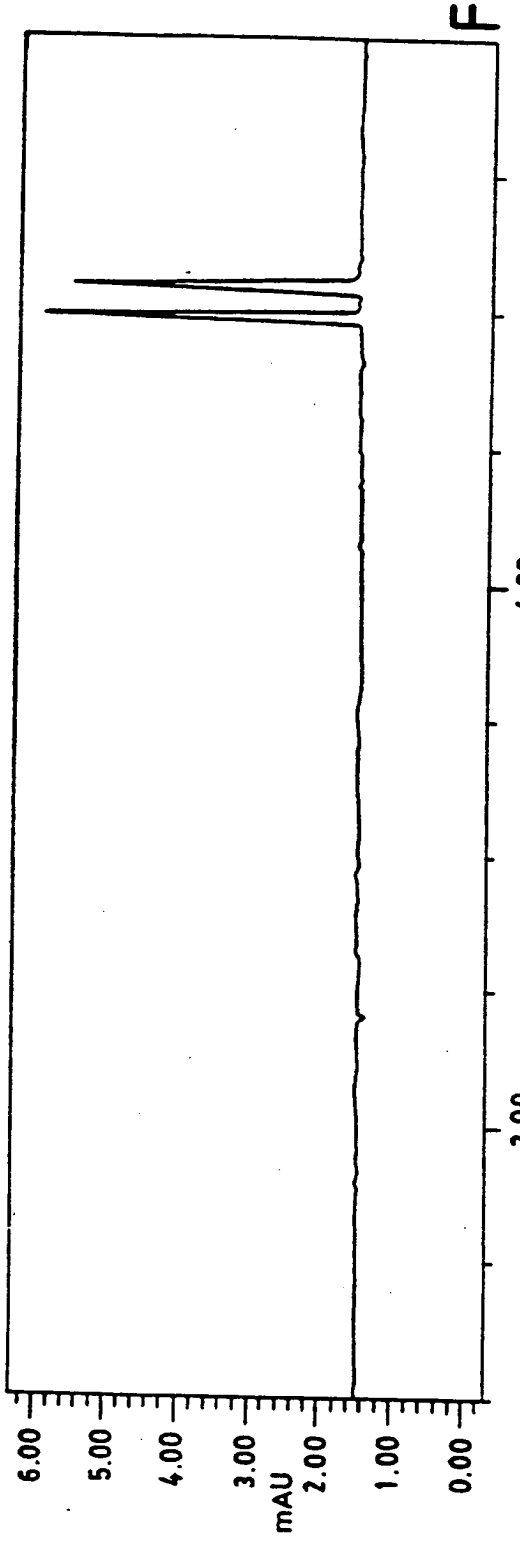


FIG.15B

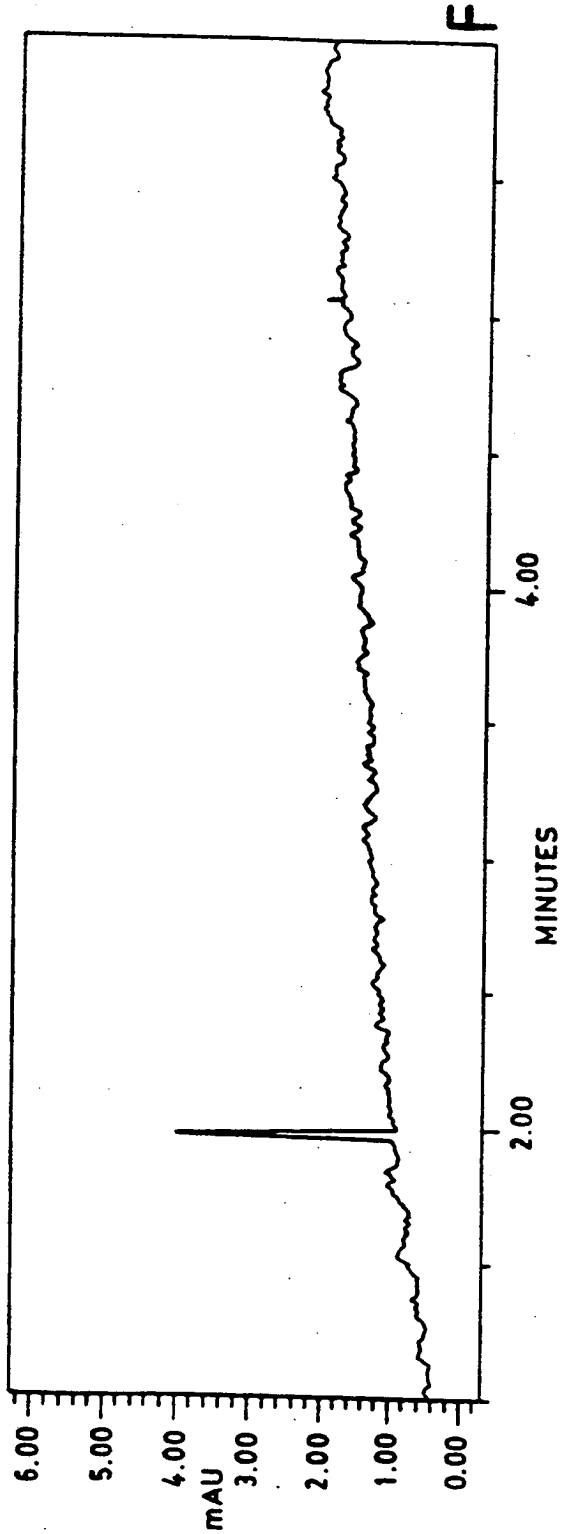


FIG.16A

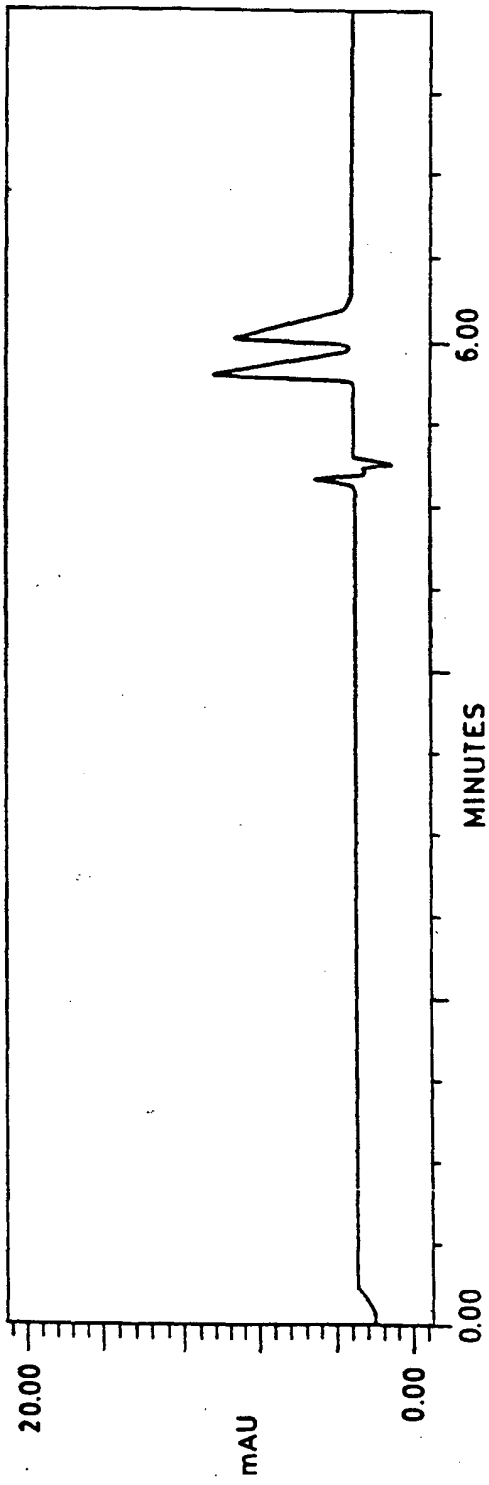
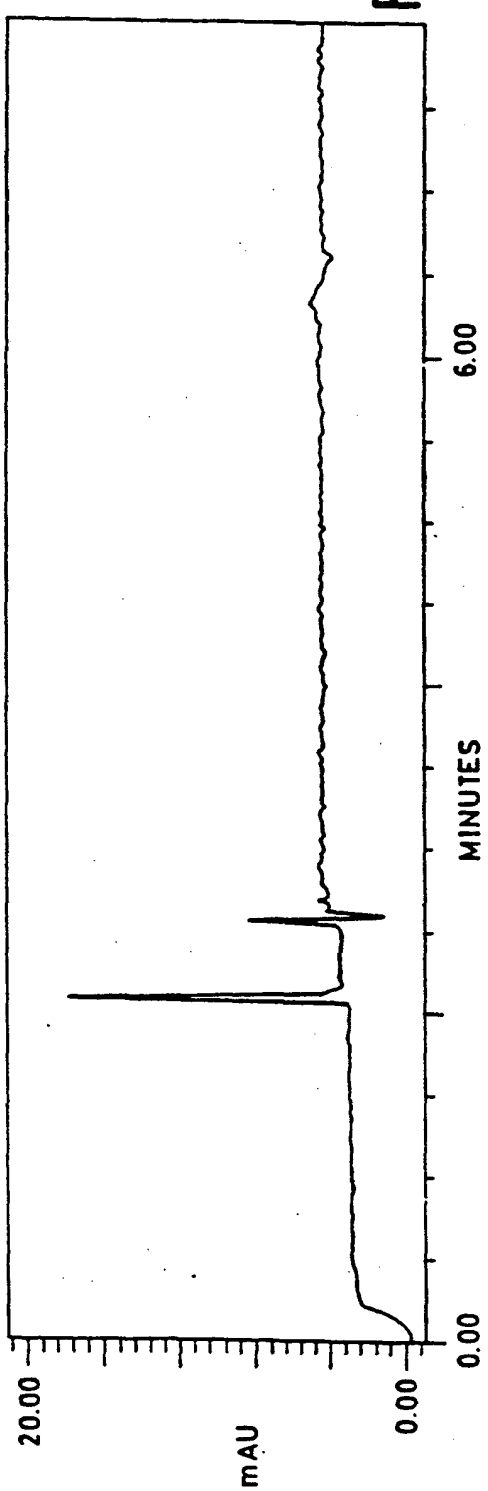


FIG.16B



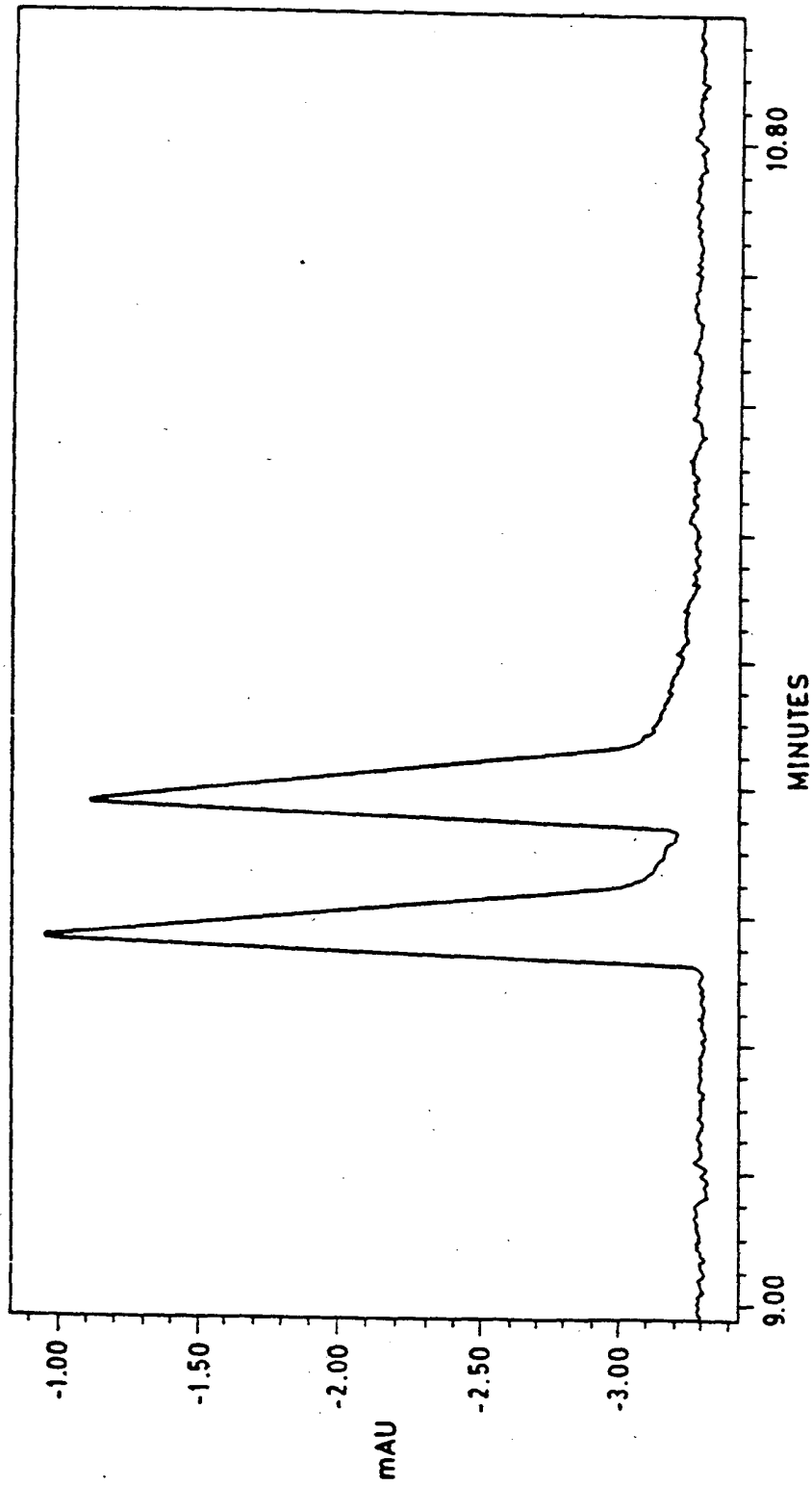


FIG. 17

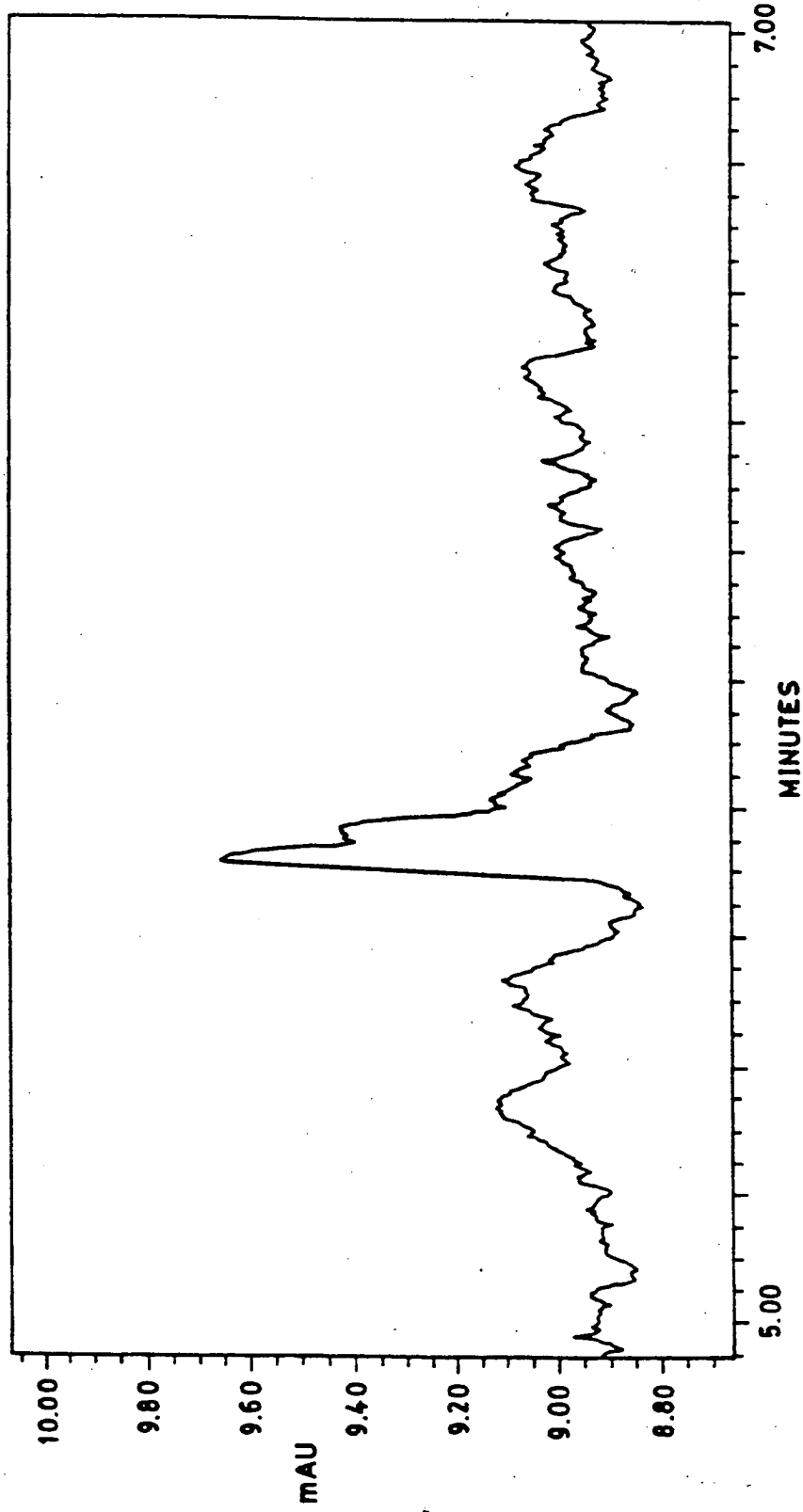


FIG. 18

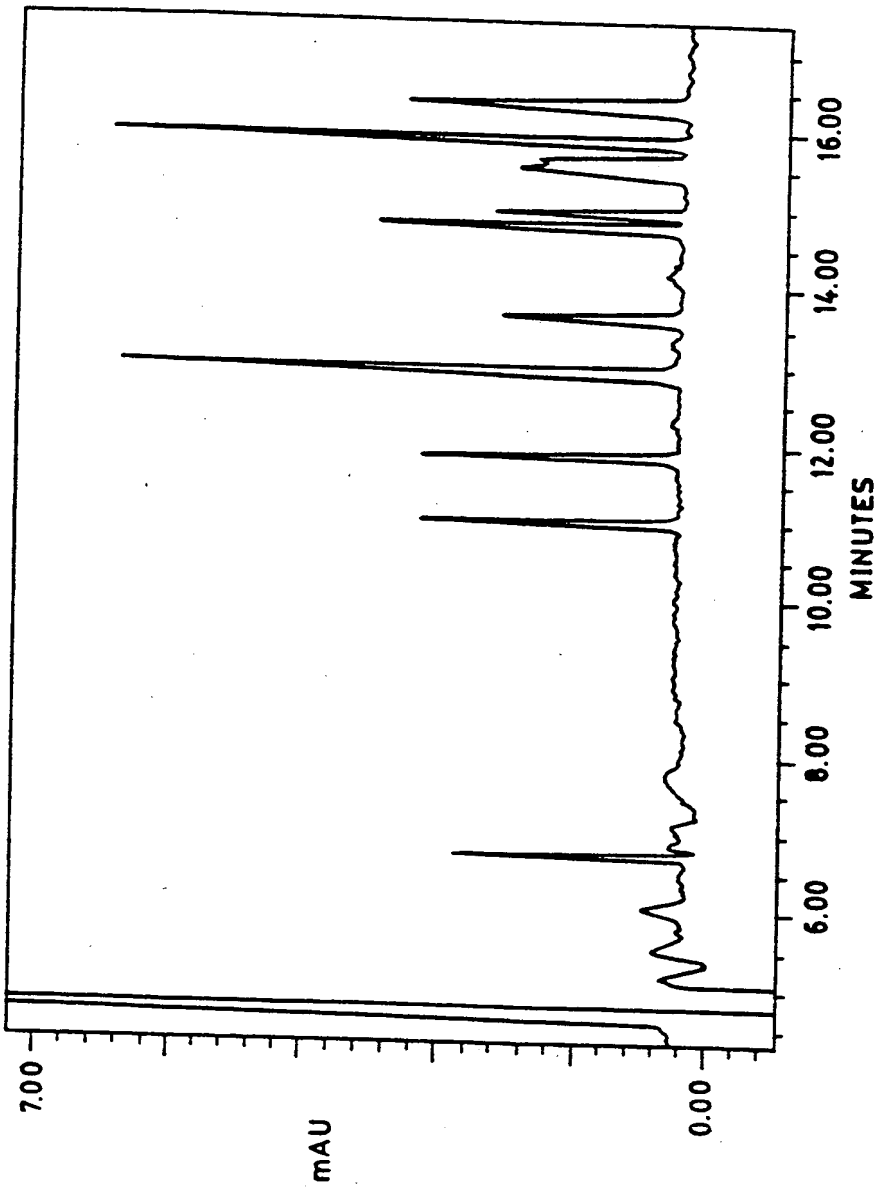


FIG. 19

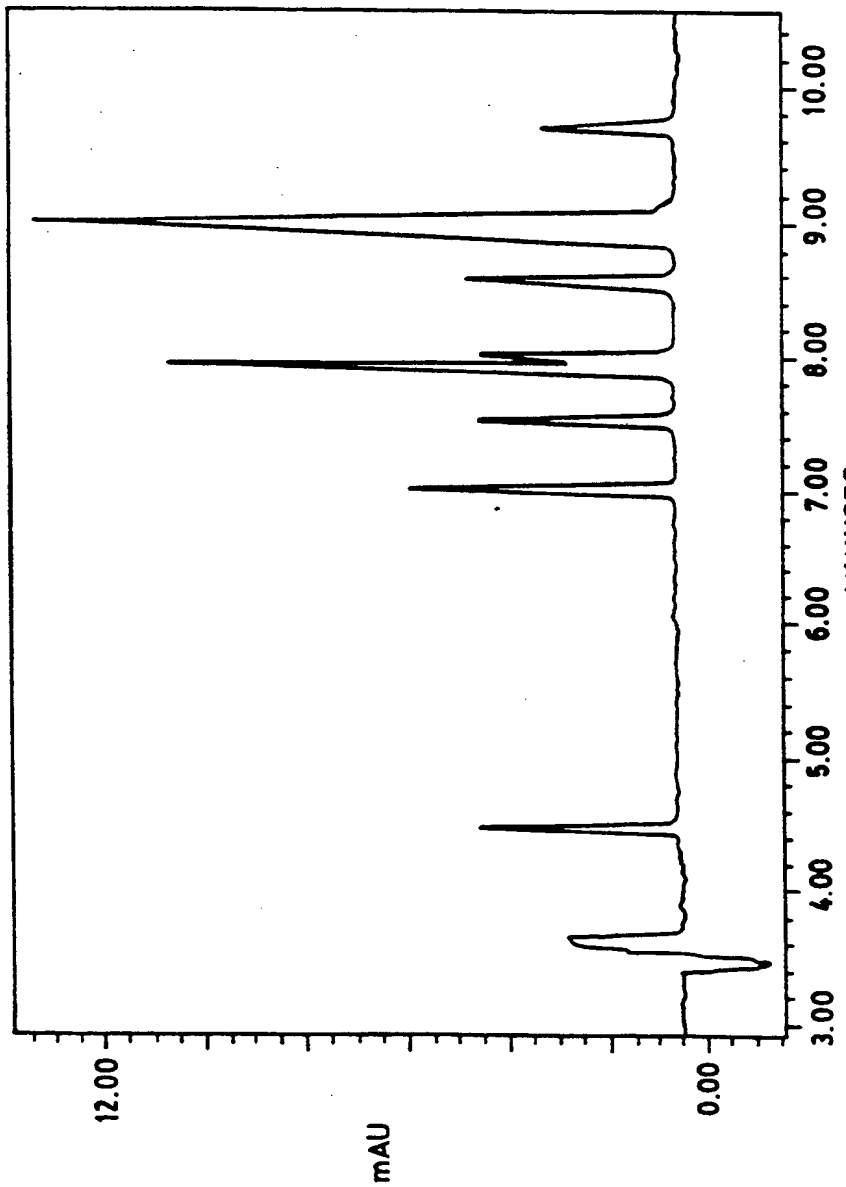


FIG. 20

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/11973

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N27/447

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHROMATOGRAPHIA, vol. 37, no. 9/10, November 1993, pages 475-481, XP000607488 T. SCHMITT: "CHARGED AND UNCHARGED CYCLODEXTRINS AS CHIRAL SELECTORS IN CAPILLARY ELECTROPHORESIS" see abstract ---	1
A	EP,A,0 292 837 (ABBOTT LABORATORIES) 30 November 1988 see claim 9 ---	1
A	WO,A,93 05389 (BECKMAN INSTRUMENTS, INC.) 18 March 1993 see claim 5 --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 1996	29. 11 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Duchatellier, M

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INTERNATIONAL SEARCH REPORT

Inte. ɔnal Application No
PCT/US 96/11973

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF CHROMATOGRAPHY, vol. 194, no. 1, 1980, AMSTERDAM, NL, pages 11-19, XP000608555 D. KANIANSKY: "ROLE OF THE CHARGE NUMBER OF THE COUNTER-IONIC CONSTITUENT IN THE SEPARATION OF ANIONS BY ISOTACHOPHORESIS" see abstract ---	1
A	CHROMATOGRAPHIA, vol. 33, no. 1/2, January 1992, pages 32-36, XP000607489 R. KUHN: "CHIRAL SEPARATIONS BY HOST-GUEST COMPLEXATION WITH CYCLODEXTRIN AND CROWN ETHER IN CAPILLARY ZONE ELECTROPHORESIS" see abstract ---	1
A	ANALYTICAL CHEMISTRY, vol. 66, no. 23, 1 December 1994, WASHINGTON, DC, US, pages 4121-4126, XP000485685 D. C. TICKLE: "GLUCOPYRANOSIDE-BASED SURFACTANTS AS PSEUDOSTATIONARY PHASES FOR CHIRAL SEPARATIONS IN CAPILLARY ELECTROPHORESIS" see page 4121 - page 4122 ---	1
A	ANALYTICAL CHEMISTRY, vol. 57, no. 4, April 1985, pages 834-841, XP000608538 S. TERABE: "ELECTROKINETIC CHROMATOGRAPHY WITH MICELLAR SOLUTION AND OPEN-TUBULAR CAPILLARY" cited in the application see the whole document -----	1

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte[illegible]nal Application No
PCT/US 96/11973

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-292837	30-11-88	CA-A-	1319007	15-06-93
		DE-A-	3883288	23-09-93
		DE-T-	3883288	09-12-93
		JP-A-	1065444	10-03-89
		US-A-	4936963	26-06-90
WO-A-9305389	18-03-93	US-A-	5259939	09-11-93
		AU-B-	657164	02-03-95
		AU-A-	2424592	05-04-93
		EP-A-	0556352	25-08-93
		JP-T-	6503179	07-04-94